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Probiotic Functional Dairy Foods and Health Claims: an Overview

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Abstract The commercial interests in functional foods containing probiotics are gaining significance in view of increasing studies on their role in digestive tract. Probiotic dairy foods containing health-promoting bacteria are important segment of functional food market. Various health benefits have been attributed to specific strains of lactic acid bacteria or food containing these probiotic cultures. Probiotic-containing foods are considered to improve general gut health and natural defenses of body, and lower blood cholesterol level. Specific probiotic microbes can alleviate or prevent diverse intestinal diarrhea-inducing disorders, cause prophylaxis of intestinal and urogenital infections, inhibit mutagenicity of intestinal contents, and reduce incidence of intestinal tumors. Recent increasing evidences on health effects of probiotics have triggered consumer interest in this category of functional foods. Rational approach needs to be applied in selection of strains for probiotic preparation to achieve required functionality. Present article focuses on some prominent probiotic candidates and criteria for their inclusion in functional food sector. Various health claims of probiotics on gastrointestinal disorders, anticarcinogenic effects, and anti-cholestrimic effects, and possible mechanistic explanations for their functionality are highlighted.

Keywords: probiotics, probiotic food, functional food, probiotic candidate, health claims, anticarcinogenic, gastrointestinal disorders, anticholesterol

Introduction

Dairy products claimed to improve health have been developed steadily during the last decades. In some countries, they are now a firmly established market segment. Today the dairy products are expected and considered to be more than just food. Besides their taste, appeal and the pleasure they provide, dairy products should add to a feeling of well-being by imparting specific health benefits and aid in preventing diseases. The probiotic foods, especially yoghurt, have made an increasingly significant impact on health-conscious people. Although many of the prophylactic and therapeutic properties of these probiotic foods on certain diseases of human beings have not yet been confirmed, there is a significant growing interest in the health aspects of probiotics worldwide (1). Probiotics are a major growing area in the food industry, reflecting the rising awareness of people for healthy, enriched, and functional foods because functional foods and/or supplements may be used in the context of a healthy lifestyle or as a means to compensate for an unhealthy lifestyle (2).

Knowledge in the field of the microbiology of fermented milk has advanced dramatically over the last decades. For example, human strains of lactobacilli and bifidobacteria are now readily available in cultures with defined levels of viable cells, and much more is known about the survival of these bacteria in various foods (3). This increasing confidence is reflected in the growing consumer demands for bio-yoghurts and other milk products that contain live cultures. The consumption of fruit and probiotic yoghurt has dramatically increased in the global market. Keeping in view of the global growing popularity of the probiotic foods, this review attempts to highlight some of the important probiotic food candidates and the health-related claims of these functional dairy foods.

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Probiotic candidates For an organism to be considered for use as a probiotic, it must be able to withstand stomach acid and bile salts, and adhere to the surface of the gastrointestinal tract to produce useful enzymes and metabolites (4). Microorganisms used in probiotic preparation need to compete with the large, well established intestinal flora of each consumer and therefore, have to fulfill the following conditions (4-6):

- Exert beneficial effect on the host, e.g. resistance to diseases.
- Be a normal inhabitant of the intestinal tract.
- Survive in the upper intestinal tract and passage through the intestinal tract in competition with the resident flora, and grow in the intestine.
- Be non-pathogenic and produce no toxins.
- Be available in multistrain preparation and present as viable cells, preferably in large numbers.
- Capable of surviving and metabolizing in the gut, e.g. resistance to acidic environment and bile acids, and able to maintain adhesiveness.
- Maintain good viability in the carrier food and remain stable and viable for long periods under storage conditions.

Besides the above criteria, probiotic cultures should improve the flavor of milk induce good acid production in fermented milk products. It is also equally important for the sake of health claims that the probiotic level in the end product be a minimum of 10^6 cfu/mL up to the last day of shelf-life (3). Viable probiotic cell must also be able to

reach the human intestine after consumption of the probiotic fermented milk product.

The probiotics currently in the market are mainly based on lactic acid bacteria (LAB), lactobacilli, bifidobacteria, and streptococci which have been shown to be important components of the gastrointestinal microflora and are relatively harmless. These bacteria have been attributed to have health-promoting properties. Therefore, there is a great interest in manipulating the composition of the intestinal flora through foods or food ingredients carrying these organisms. The majority of probiotic yoghurt contains *Bifidus* and/or *L. acidophilus* cultures. These LAB make up a big portion of the present probiotic cultures (7, 8).

Probiotics may consist of a single strain or up to eight strains. The attraction of multistrain preparations is that they have a wide range of activities (5). The species that are currently being used in probiotic preparations are *L. acidophilus, L. casei, L. helveticus, L. lactis, L. salivarius, L. plantarum, Enterococus faecium, E. faecalis, bifidobactrium* spp. and *E. coli. L. delbrueckii* subsp. *Bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* are yoghurt organisms included in the probiotic list based on the therapeutic claims made for yoghurt. However, the ability of these organisms to colonize the gut is extremely doubtful because they are not intestinal strains (7, 8).

Yoghurts fermented with cultures of intestinal origin, so called bio-yoghurts have been recently developed. A new generation of starter cultures and bio-cultures has been employed to produce a wide range of fermented milk products including yoghurt and fermented milk drinks such as Yakult and acidophilus milk. The popularity of these starter cultures have increased due to the health properties they are purported to impart. In addition, their fermentation stops at a higher pH than those of traditional cultures (around pH 4.3), and they do not sour during storage, which results in milder tasting products and enables lower levels of sweetness to be used in products with added fruits (9).

The culture selection is now based on more specific criteria, and recently the potential for promoting a healthy digestive system in humans has become a priority. For a strain to be claimed as probiotic, it must have a competitive advantage in the human gut ecosystem. Probiotic organisms are incorporated in yoghurt and dairy desserts with the intention of replenishing deficiencies in the composition of the gut microflora with a view to produce beneficial effects in the consumer (10).

The presently available nutrish cultures are based on combinations of *L. acidophilus* and *Bifidobacterium* with thermophilic or mesophilic aromatic single strain cultures. The most successful nutrish cultures have been the *Acidophilus* and *Bifidobacterium* (AB) cultures, which give high viscosity, low post fermentation, and a very mild taste to the end product. These cultures also maintain high concentrations of AB even at the last day of the shelf life. Single strain cultures of BB-11, a *B. bifidum* strain, and BB-46, a *B. longum* strain, have also been introduced. The culture *L. casei* is also included in the nutrish range of direct vat set (DVS) cultures due its growing demand in various fermented milk products (Yakult, ViFit, *etc.*). Based upon the development of probiotic single strain culture concept, several new blends of ABC cultures have been developed *(L. acidophilus, Bifidobacteria,* and *L. casei*). These blends produce fermented milk products with a new mild taste, low post acidification, and high viscosity (7, 8).

The development of defined strain starter cultures for direct inoculation (DVS) has provided the dairy industry with a very useful tool in product formulation because the strains allow better overall product quality as well as economic production. These types of cultures secure fermentation without contamination, particularly for very slow-growing probiotic bacteria. Because they are inoculated directly into the process milk, the newly developed mesophilic range of cultures (eXact^{TM}), which are available in DVS from Christian Hansen, Denmark, for instant inoculation, offer ease and convenience. They provide remarkable aroma and texture to the end product (9). A new strain, *L. casei* CRL 431 isolated from the feces of a healthy child has been subjected to several studies *in vitro* as well as *in vivo* using both animal and human models. The probiotic properties of this strain have been compared to a large number of other lactic acid *bacteria* and were found to be superior to most of the tested organisms in a number of physiological criteria (11). *In vitro* studies of *L. casei* CRL 431 have revealed that they have strong inhibitory effects towards enteropathogens such as *E. coli* and *Shigella sonnei* (12, 13).

Health claims of probiotics Stable intestinal microflora helps develop resistance to infections and, in general, promotes improved health in humans and animals (7). The large intestine is the most densely colonized part of the human gastro intestinal tract. The composition and numbers of different kinds of bacteria present in the large intestine of a person appear to be relatively stable over a period of time. However, numerous factors affect the composition of an individual intestinal flora. Such factors result in a decline in the number of beneficial bacteria such as bifidobacteria, and an increase in harmful bacteria such as *E. coli, Clostridium perfringens,* and *Staphylococcus aureus.* (5). It is well established that *L. acidophilus* and *Bifidobacterium* species possess antimicrobial properties against intestinal pathogens, as demonstrated *in vivo* against a variety of undesirable bacteria including *E. coli, Salmonella, Shigella, S. aureus,* and *Vibrio* species. The inhibition may be due to the complex interaction of factors including lactic acid, acetic acid, and antibiotics (14).

Although the results of *in vitro* experiments do not confirm antimicrobial activity in the intestine, it is likely that carefully selected strains of human origin that are bile-tolerant and have the potential to colonize the bowel will assist in the control of intestinal disorders. Inhibition of pathogen growth *in vitro* by lactobacilli has stimulated considerable interest in using these microorganisms for prophylactic and therapeutic means in treating gastrointestinal diseases. Some of the established health benefits of probiotics foods (6, 8, 15, 16) are:

- Control of intestinal pathogens and increased resistance to infection
- Alleviating several types of diarrhea diseases

· Improved lactose digestion
· Reduction in the serum cholesterol level
· Anticarcinogenic activity and prevention of cancer

Other health benefits minor significance include improved absorption of some nutrients and intestinal mobility, inactivation of enterotoxins, alleviation of constipation, and relieving of vaginitis (6). Most health aspects claimed are clearly linked to the perceived properties of the probiotic cultures, primarily their ability to stabilize the intestinal flora of humans and the prevention of constipation and diarrhoea. These cultures have been shown to prevent travelers' diarrhea (17). Reduction of lactose-intolerance by the consumption of fermented milk products has been very well documented (18, 19). Although some indication of their positive influence does exist with respect to other health claims, the present scientific evidence is not sufficient to positively support the health promotion aspects of probiotic bacteria in these areas (8, 20). According to the article on the research status of fermented milk products in Japan, as reviewed by Akiyoshi Hosono (21), LAB and bifidobacteria could play important roles in preserving human health by controlling the intestinal microflora capable of producing toxic effects on the host. Their importance has also now come to be recognized in the field of preventive medicine. Some of the established important health claims of probiotics have been briefly discussed earlier in this review.

Anticarcinogenic effects Cancer is a complex transformation of normal cells into pathological cells (22). Several investigations have shown that dietary intake of fermented milk products, containing LAB alters the intestinal microecology of the host. Consumption of fermented milk products may reduce the diet-associated risk of carcinogenesis either by the reduction of carcinogen itself or by reducing the levels of enzymes that promote the conversion of precarcinogens into carcinogens (23). Consumption of fermented milk products containing *L. acidophilus* has been shown to significantly reduce the number of fecal putrefactive bacteria in the intestine (6), suggesting that the supplementation of *L. acidophilus* reduces the level of putrefactive organisms such as coli forms and increases that of lactobacilli. This may have a beneficial effect on the intestinal microecology by suppressing the putrefactive organisms that are possibly involved in the production of tumor promoters and putative precarcinogens. Animal and human studies indicate that feeding certain lactic cultures can result in a decrease of fecal enzymes that may be involved in the formation of carcinogens. However, it is doubtful whether a reduction in these enzyme activities affects cancer rates in humans. Indeed, the origin of cancer-causing carcinogens in humans is still unknown. At present, results of epidemiologic studies do not appear to support those of experimental studies on lactobacilli and cancer prevention (6).

Although there have been conflicting reports regarding the ability of fermented milk products to prevent and/or inhibit carcinogenesis, the probable mechanisms suggested for the mode of action of LAB in preventing cancer or acting as an anticarcinogen are (7, 14, 24, 25):
· Growth suppression of the intestinal microflora incriminated in the production of putative carcinogens
· Production of antitumurigenic or antimutagenic compounds
· Alteration of physiological conditions (such as pH)
· Alteration of the metabolic activity of intestinal flora and the action of bile acids, causing quantitative and/or qualitative changes in the bile acid-degrading bacteria
· Reduction in the levels of fecal bacterial enzymes such as β-glucosidase, β-glucuronidase, nitroreductase, and azoreductase, considered to be carcinogenesis-causing factors
· Metabolical conversion, degradation, and absorption of carcinogenic compounds
· Stimulation of host immunity in the gastro-intestinal tract
· Assimilation and detoxification of dietary, endogenous and intestinal flora generating toxic compounds
· Deconjugation of bile acids, which are considered to have a role in carcinogenesis

A study of animal experiments demonstrated that yoghurt induces a significant reduction in the inflammatory immune response and inhibits tumor growth. The yoghurt exerts antitumor activity by reducing the inflammatory immune response, which was markedly increased when the carcinogen was administered (26). The anti-mutagenic activities of live and killed cells of different strains of *L. acidophilus*, *Bifidobacterium,* and organic acids generally produced by these probiotic bacteria have been demonstrated using potent chemical mutagens (27). Dietary administration of lyophilized cultures of *B. longum* strongly suppressed colon and mammary tumor developments (28). The anti-proliferation effects on a number of tumor lines by two lactic acid bacteria strains isolated from human feces have been reported by Zabala *et al.* (29). Although a number of reports suggest that certain LAB, including *L. acidophilus* and *Bifidobacterium* species may have an anticancer role, scientific evidence with respect to human subjects is lacking. Several *in vitro* studies revealed that fermented milk products containing lactic acid bacteria inhibit mutagenic activity. However, there is a danger in extrapolating the results of *in vitro* experiments to health claims for humans, because little is known about the fate of the mutagens in the human gastrointestinal system (6).

Gastro-intestinal disorders Gastrointestinal infection sometimes may lead to diarrhea as a result of changes in the intestinal flora caused by an individual pathogen (i.e infective bacteria). In healthy subjects, various microbial species of the intestinal flora are present in different proportions, and together form a natural ecological barrier against infective bacteria (30). The organisms such as bifidobacteria and lactobacilli are considered to have antagonistic effects towards undesirable microorganisms (18). The beneficial effects of these organisms have been proven in regard to traveler's diarrhea (31, 32), antibiotic-associated diarrhea, and rotavirus shedding and infection (33). Placebo-controlled studies have shown that LAB can prevent antibiotic-associated diarrhea and acute infantitis diarrhea (34).

The protective effect of lactic acid bacteria against infectious organisms may be due to lowering of pH, adhesion to the intestinal valve, thereby preventing

colonization by pathogens, competition for nutrients, production of antibacterial substances, and neutralization of toxins (18, 35). *Lactobacillus* GG (LGG), a variant of *L. casei* subsp. *rhamnosus*, is one of the most extensively studied probiotic organisms and has proven benefits in reducing the severity and duration of diarrhea. Consumption of foods containing LGG may shorten the course of rotavirus infection-causing diarrhea, traveler's diarrhea, and antibiotic-associated diarrhea (33). Fermented milk containing *L. acidophilus* and *L. casei* were fed to two groups of human subjects to evaluate the protective effect of this product with respect to diarrhea. The result demonstrated that fermented milk could be used for the prevention of gastrointestinal disorders in high-risk children (36).

An *in vitro* study demonstrated that *L. acidophilus* M92 possesses antimicrobial activity against enteropathogenic, spore-forming, and fungal microorganisms. The study also indicated that *L. acidophillus* M92 has a chance of survival in the gastrointestinal tract (37). Antibiotic treatment is often accompanied by diarrhea and other gastrointestinal side effects. Feeding of LGG yoghurt to antibiotic-treated persons (using erythromycin) resulted in reduced diarrhea and other side effects such as abdominal distress, stomach pain, and flatulence (38), as demonstrated by the studies of Siltonen *et al.* (39) on volunteer subjects provided with *Lactobacillus* GG yoghurt after antibiotic treatment in comparision with control subjects given only the pasteurized yoghurt. A human study showed *L. casei* CRL 431 has great potential in curing diarrhea in mal-nourished children. Children from the slum area of Tucuman, Argentina suffering from severe diarrhea were given milk treated with *L. casei* CRL 431. Within 4 days treatment with the fermented milk containing these cultures, which otherwise could not be cured by ordinary medical treatment, eliminated diarrhea within 4 days (40). Sakamoto *et al.* (41) demonstrated that *L. gasseri* is effective both in suppressing *H. pylori* and reducing mucosal inflammation in 31 human subjects, suggesting that probiotics have positive effects in controlling the gastrointestinal-related disorders.

Reduction in serum cholesterol levels Elevated cholesterol is considered to be the major risk factor of coronary heart disease. It is evident from the medical reports that hypercholesteremia is closely associated with coronary heart disease, one of the leading causes of death globally (42). Reports claiming that fermented milk products may lower plasma cholesterol levels have stimulated much debate throughout the world, although considerable research is needed to clearly establish the benefits of *Lactobacillus* in lowering the plasma cholesterol levels (14). Recently, interest has been focused on the potential of fermented milk products in reducing the blood cholesterol levels, particularly the low density lipoprotein (LDL) fraction. Consumption of fermented milk products correlates with a reduction of about 4.3 to 5.6% in LDL cholesterol (15, 18). A 10% reduction has been observed by the consumption of a yoghurt-like product containing *E. faecium* for 6 weeks (43). On the other hand, a study conducted by Richelsen *et al.* (44) revealed that low fat milk products may have generalized cholesterol-lowering properties. Possible mechanisms suggested for the hypocholesterolaemic effects of fermented milk products are (43, 45, 46):

- Cholesterol-lowering factors of LAB, which may inhibit hepatic cholesterol synthesis
- Certain strains of bacteria can assimilate cholesterol in the presence of bile acids
- Deconjugation of bile acids in the upper part of the gut by certain bacteria in fermented milk products

A study conducted in the Netherlands on human subjects revealed that feeding yoghurt containing *L. acidophilus* significantly reduced total serum cholesterol, LDL, and the ratio of LDL and high density lipoprotein (HDL). The study involved comparison of 30 men fed daily with 125 mL yoghurt containing two strains of *L. acidophilus* for three times daily with each meal for 3 weeks with the control subjects not given yoghurt (47). In an animal model experiment, feeding the *Bifidus* yoghurt to albino rats reduced both total and LDL cholesterol levels, suggesting it may have potential value in human subjects (48). The administration of *L. reutteri* CRL 1098 to hypercholesterolemic mice at 10^4 cells/day for 7 days decreased total cholesterol level by 38%. At this concentration, a 40% reduction in triglycerides and a 20% increase in the ratio of HDL to LDL were found without bacterial translocation of the native microflora into the spleen and liver (49). Consumption of 450 mL probiotic milk products possessing Causido R cultures daily for 8 weeks by overweight subjects resulted in reduction of LDL-cholesterol and increased fibrinogen content (50). Kiessling *et al.* (51) reported that long-term consumption of probiotic yoghurt (yogurt enriched with *L. acidophilus* 145, *B. longum* 913 and 1% oligofructose, i.e. symbiotic) for over 6 months increases HDL cholesterol and led to improvement of the LDL/HDL cholesterol ratio in comparison to control subjects given plain yogurt. Similar results were also observed with the long-term daily consumption of 300 g yoghurt for 21 weeks (control and symbiotic) (51). A significant lowering of total serum cholesterol, LDL cholesterol, and triglycerides, with no change in HDL cholesterol was observed by feeding milk products fermented with the *B. longum* strain BL1 to rats and healthy adult male volunteers (52). It has been suggested that probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut resulting in a decrease in the systemic levels of blood lipids by inhibiting the hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver. Furthermore, some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts, thereby affecting the metabolism of cholesterol, or by directly assimilating cholesterol (53). These animal and human studies suggest that consumption of dairy products fermented with appropriate strain(s) of LAB and bifidobacteria could result in a moderate cholesterol-lowering effects.

Legal aspects: regulations Apart from the scientific knowledge, the establishment of health claims of probiotic functional dairy foods must also be firmly based upon legal regulations. The special efficacy that probiotic

functional dairy foods possess must be strictly verified nutrition-wise due to the restrictive national regulations, pharmacologically in pharmacy due to legal restraints, and in food applications in accordance with the food law regulations. Although used for a long time in food specimens and pharmaceuticals, the beneficial effects of probiotic functional dairy foods have probably been best claimed in USA. The FDA of USA, the world's largest functional food-producing country, permits the use of the health claims of functional food including probiotic functional dairy foods in accordance with the Nutrition Labeling and Education Act (54). From Japan's perspective, foods that exhibit functional properties are classified as functional foods in the form of specified health use (FOSHU). Effective from April 2001, Japan's Ministry of Health, Labor and Welfare permits the claim of specific health benefits by probiotic functional dairy foods, and as of September 27, 2004, a total of 454 products have been approved with fermented milk comprising 78% of FOSHU (55). On the contrary, in European Union, although the market share of probiotic dairy foods is increasing, debate still exists on the definition of products using probiotics, scientific evidences that can prove the health benefits, extent of their use, and the existence of satisfactory bioassays (56). In Korea, various types of functional foods including dairy products are now on the market, and to meet this situation, functional health food act has been introduced on August 26, 2003 by Ministry of Health and Welfare. However, advertisement of the health claim of probiotic functional dairy foods is still prohibited.

Conclusion

The concept of probiotics is based on the knowledge that the intestinal microflora are beneficial to human beings, providing protection against various diseases. Increasing evidences in the scientific literature point out that the consumption of yoghurt, in particular fermented milk containing *L. acidophilus* and *Bifidobacterium* species, offers considerable health benefits to the consumer, in addition to improved flavor and nutrition. However, many of the claims made regarding their prophylactic properties for certain human diseases have yet to be substantiated. Although some of these scientific claims are valid, the public may misinterpret such messages. Therefore, probiotic effects should be examined using a multidisplinary approach for better understanding, as each probiotic strain possesses different beneficial effects for specific purposes.

For starter culture production of probiotics, it is important to constantly regulate bacterial cultures to meet the changing demands of consumers. Hence, there is a need for a sustained effort to combine the skills of microbiologists, food technologists, and clinicians. Undoubtedly, gut flora can protect the host against several intestinal diseases. Once the prophylactic effect of probiotics are well established with respect to claimed health benefits, probiotics are sure to gain a firm footing in the health food sector of the market, and will certainly become a major portion of functional food sector.

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