MINIREVIEW

Probiotics, their health benefits and applications for developing healthier foods: a review

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Abstract

In the industrialized world, functional foods have become a part of an everyday diet and are demonstrated to offer potential health benefits beyond the widely accepted nutritional effects. Currently, the most important and frequently used functional food compounds are probiotics and prebiotics, or they are collectively known as 'synbiotics'. Moreover, with an already healthy image, dairy products appear to be an excellent mean for inventing nutritious foods. Such probiotic dairy foods beneficially affect the host by improving survival and implantation of live microbial dietary supplements in the gastrointestinal flora, by selectively stimulating the growth or activating the catabolism of one or a limited number of health-promoting bacteria in the intestinal tract, and by improving the gastrointestinal tract's microbial balance. Hence, the paper reviews the current scenario of probiotics and their prospective potential applications for functional foods for better health and nutrition of the society.

Introduction

Probiotics are defined as 'live microorganisms which when administered in adequate amount confer health benefits to the host' (FAO/WHO, 2002). Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotics were originally used to improve the health of both animals and humans through the modulation of the intestinal microbiota. At present, several well-characterized strains of *Lactobacilli* and *Bifidobacteria* are available for human use to reduce the risk of gastrointestinal (GI) infections or treat such infections (Salminen *et al.*, 2005). Some of the beneficial effects of probiotic consumption include improvement of intestinal health by the regulation of microbiota, and stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, and reducing the risk of certain other diseases (Fig. 1; Kumar *et al.*, 2009a, b, 2010, 2011a, b; Nagpal *et al.*, 2007, 2010, 2011; Yadav *et al.*, 2007a, b, 2008). The primary clinical interest in the application of probiotics has been in the prevention of and treatment for GI infections and diseases (Parvez *et al.*,

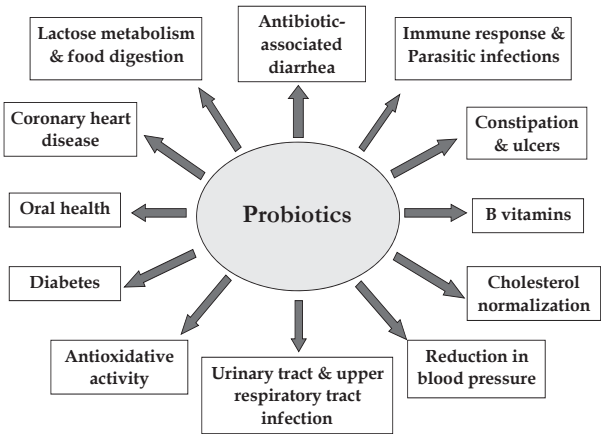

Fig. 1. Projected prospective health attributes of probiotics.

2006). Gut microbiota deviations have been associated with enhanced risk of specific diseases; therefore, modulation of an unbalanced indigenous microbiota forms the rationale of probiotic therapy (Turnbaugh *et al.*, 2006). Also, the development of adjuvant or alternative therapies based on bacterial replacement is becoming important owing to the rapid emergence of antibiotic-resistant pathogenic strains and the adverse consequences of antibiotic therapies on the protective flora, which enhances the risk of infection (Forestier *et al.*, 2001). However, the use of probiotics should be further investigated for their benefits and possible side effects, if any. As the knowledge about intestinal microbiota, nutrition, immunity, and genetics in health and disease has increased in the past years, such information could certainly help to develop new probiotic strains with disease-specific functions and could also facilitate the understanding of when to use probiotics and how they affect specific pathological states. However, it is important that the probiotic strains for human use should undergo animal studies followed by human clinical trials in order to authenticate the suitability, safety, and benefits of probiotics for human consumption and development of functional foods.

Properties essential for effective and successful probiotics

It is of utmost importance that the probiotic strain survives the site where it is presumed to be active. For maximum activity, the strain should be able to proliferate and colonize at this specific location. Besides, it should also be tolerated by the immune system. It should not be pathogenic, allergic, or mutagenic/carcinogenic (Toma & Pokrotnieks, 2006; Ohashi & Ushida, 2009). Probiotics for human should have 'generally regarded as safe' status, with a proven low risk of inducing or being associated with the etiology of disease. The probiotic organisms should preferably be of human origin (Collins *et al.*, 1998), must be able to survive and grow in the *in vivo* conditions of the desired site of administration, and thus must be able to tolerate low pH and high concentration of both conjugated and deconjugated bile acids. For successful application in foods, the probiotic used should also be technologically compatible with the food-manufacturing process. In addition to that, the foods containing the probiotic bacteria must maintain the characteristic sensory attributes of the traditional food.

Potential attributes and benefits of probiotics

It is now an established fact that the indigenous microbial communities is host specific, location specific, very complex in composition and has beneficial properties to the host. However, it is not precisely known which species of microorganisms play the principal part in these beneficial properties. Some major health benefits of probiotics and their proposed mechanisms are illustrated in Table 1. Several probiotic bacteria have been introduced in the market, and the range of products in which probiotic bacteria are added is increasing (Table 2). Some of the major health attributes of probiotics are discussed in the following sections.

Antimicrobial properties

The intestinal microbial community is a complex ecosystem, and introducing new organisms into this highly competitive environment is difficult. Thus, organisms that can produce a product that inhibits the growth of existing organisms have a characteristic advantage. The ability of probiotics to establish in the GI tract is enhanced by their ability to eliminate competitors. Some antimicrobials with producer organisms are enlisted in Table 3. In different studies on humans and animals, beneficial microorganisms are used to improve the colonization resistance on body surfaces, such as GI, the urogenital, and the respiratory tract. *Bifidobacteria* produce acetic and lactic acids in a molar ratio of 3 : 2 (Desjardins & Roy, 1990). *Lactobacillus acidophilus* and *Lactobacillus casei* produce lactic acid as the main end product of fermentation. In addition to lactic and acetic acids, probiotic organisms produce other acids, such as hippuric and citric acid. Lactic acid bacteria also produce hydrogen peroxide, diacetyl, and bacteriocin as antimicrobial substances. These inhibitory substances create antagonistic environments for foodborne pathogens and spoilage organisms. Yoghurt bacteria are reported to produce bacteriocin against probiotic bacteria and vice versa (Dave & Shah, 1997).

Anticarcinogenic properties

Goldin & Gorbach (1980) reported that the introduction of *L. acidophilus* into the diet lowers the incidence of chemically induced colon tumors in rats. Later, the same authors also suggested that diet and antibiotics can lower the generation of carcinogens in the colon and reduce chemically induced tumors (Goldin & Gorbach, 1984). These effects appear to be mediated through the intestinal microbial communities. A possible mechanism for these anticancer effects relies on inhibiting intestinal bacterial enzymes that convert procarcinogens to more proximal carcinogens (Kumar *et al.*, 2011a, b). This approach can be expanded in the future by testing probiotics for their ability to inhibit the growth of organisms normally found

Table 1. Health benefits of probiotic bacteria to the host, and speculated mechanisms involved

Health benefits	Proposed mechanisms involved
Resistance to enteric pathogens	Antagonism activity Adjuvant effect increasing antibody production Systemic immune effect Colonization resistance Limiting access of enteric pathogens (pH, bacteriocins/defensins, antimicrobial peptides, lactic acid production, and toxic oxygen metabolites)
Aid in lactose digestion	Bacterial lactase acts on lactose in the small intestine
Small bowel bacterial overgrowth	Lactobacilli influence the activity of overgrowth flora, decreasing toxic metabolite production Normalization of a small bowel microbial community Antibacterial characteristics
Immune system modulation	Strengthening of nonspecific and antigen-specific defense against infection and tumors Adjuvant effect in antigen-specific immune responses Regulating/influencing Th1/Th2 cells, production of anti-inflammatory cytokines Decreased release of toxic N-metabolites
Anticolon cancer effect	Antimutagenic activity Detoxification of carcinogenic metabolites Alteration in pro-cancerous enzymatic activity of colonic microorganisms Stimulation of immune function Influence on bile salt concentration
Decreased detoxification/ excretion of toxic microbial metabolites	Increased bifidobacterial cell counts and shift from a preferable protein- to carbohydrate-metabolizing microbial community, less toxic and for putrefactive metabolites, improvements of hepatic encephalopathy after the administration of bifidobacteria and lactulose
Allergy	Prevention of antigen translocation into blood stream Prevent excessive immunologic responses to increased amount of antigen stimulation of the gut
Blood lipids, heart disease	Assimilation of cholesterol by bacterial cell Alteration in the activity of BSH enzyme Antioxidative effect
Antihypertensive effect	Bacterial peptidase action on milk protein results in antihypertensive tripeptides Cell wall components act as ACE inhibitors
Urogenital Infections	Adhesion to urinary and vaginal tract cells Competitive exclusion Inhibitor production (H_2O_2, biosurfactants)
Infection caused by *Helicobacter pylori*	Competitive colonization Inhibition of growth and adhesion to mucosal cells, decrease in gastric *H. pylori* concentration
Hepatic encephalopathy	Competitive exclusion or inhibition of urease-producing gut flora
Neutralization of dietary carcinogens	Production of butyric acid neutralizes the activity of dietary carcinogens
NEC (necrotic inflammation of the distal small intestine)	Decrease in TLRs and signaling molecules and increase in negative regulations Reduction in the IL-8 response
Rotaviral gastroenteritis	Increased IgA response to the virus
Inflammatory bowel diseases, type I diabetes	Enhancement of mucosal barrier function
Crohn's disease	Reduction in proinflammatory cytokines including TNFα, reduction in the number of CD4 cells as well as TNFα expression among intraepithelial lymphocytes
Caries gingivitis	Reduction in gingivitis by *L. reuteri,* affects on streptococcus mutants, colonization of the teeth surface by lactobacilli Less carries after the ingestion of living or oral vaccination with heat-killed lactobacilli
Enhanced nutrient value	Vitamin and cofactor production

in the flora that have high activities of enzymes such as β-glucuronidase (Reddy, 1999), nitroreductase, azoreductase, and β-glycosidase or the capability for nitrosation.

The sixth most commonly diagnosed cancer in the world is hepatitis B virus. Consumption of foods, contaminated with aflatoxins, is also established causes of liver cancer. Aflatoxin B1 (AFB1) causes characteristic genetic changes in the p53 tumor suppressor gene and ras protooncogenes. Some probiotic bacterial strains have been successfully shown to bind and neutralize AFB1 *in vivo* and thus reduce the bioabsorption of the toxin from the gut (Haskard *et al.*, 2000; Kumar *et al.*, 2011a, b). Addition of probiotic *Bifidobacterium longum* to the diet of rats has been shown to exert a strong antitumor activity on colonic mucosa by reducing the expression level of ras-p21 expression and cell proliferation (Reddy, 1998). *Lactobacillus* GG administration determined the up- and downregulation of 334 and 92 genes, respectively, by

Table 2. Some commercial probiotic strains used by various industries

Strains	Source
L. acidophilus LA-1 *L. paracasei* CRL 431 *B. lactis* Bb-12	Chr. Hansen (Horsholm, Denmark)
L. casei Shirota *B. breve* strain Yakult	Yakult (Tokyo, Japan)
L. acidophilus SBT-2062 *B. longum* SBT-2928	Snow Brand Milk Products Co., Ltd (Tokyo, Japan)
L. acidophilus R0011 *L. rhamnosus* R0052	Institut Rosell (Montreal, Canada)
L. acidophilus NCFM	Rhodia, Inc. (Madison, WI)
L. acidophilus DDS-1	Nebraska Cultures, Inc. (Lincoln, NE)
L. casei DN014001 (Immunitas)	Danone Le Plessis-Robinson (Paris, France)
L. fermentum RC-14 *L. rhamnosus* GR-1	Urex Biotech Inc. (London, Ontario, Canada)
L. johnsonii La1 (same as Lj1)	Nestlé (Lausanne, Switzerland)
L. plantarum 299V *L. Rhamnosus* 271	Probi AB (Lund, Sweden)
L. reuteri SD2112 (same as MM2)	BioGaia (Raleigh, NC)
L. rhamnosus GG	Valio Dairy (Helsinki, Finland)
L. rhamnosus LB21 *Lactococcus lactis* L1A	Essum AB (Umeå, Sweden)
L. salivarius UCC118	University College (Cork, Ireland)
B. longum BB536	Morinaga Milk Industry Co., Ltd (Zama-City, Japan)
L. delbrueckii subsp. *bulgaricus 2038*	Meiji Milk Products (Tokyo, Japan)
L. acidophilus LB	Lacteol Laboratory (Houdan, France)
L. paracasei F19	Arla Dairy (Stockholm, Sweden)
L. crispatus CTV05 *L. casei DN 114* *S. boulardii*	Gynelogix, Boulder, CO Danone, Paris, France Biocodex Inc. (Seattle, WA)
B. lactis HN019 (DR10)	New Zealand Dairy Board

Table 3. Antimicrobial substances produced by probiotic bacteria (Fuller, 1992)

Probiotic	Compound
Lactobacillus GG	Wide-spectrum antibiotic
L. acidophilus	Acidolin, Acidophilin, Lactocidin, Lactocin B
L. delbrueckii ssp. *bulgaricus*	Bulgarican
L. plantarum	Lactolin
L. brevis	Lactobacillin, Lactobrevin
L. reuteri	Reuterin
L. sake L45, L. sake Lb706	Lactocin S, Sakacin A
L. johnsonii	Lactocin F
L. helveticus	Helveticin J
L. cremoris	Diplococin
Lactococcus lactis	Nisin, Lactostrepsin, Lactocin, Lacticin
Pediococcus pentosaceous, P. acidilactis	Pediocin
S. thermophilus	Streptophilin
Enterococcus faecium DPC1146	Enterocin 1146

affecting the expression of genes involved in immune response and inflammation [transforming growth factor-beta (TGF-β) and tumor necrosis factor (TNF) family members, cytokines, nitric oxide synthase 1, defensin alpha-1], apoptosis, cell growth and cell differentiation (cyclins and caspases, oncogenes), cell–cell signaling (intracellular adhesion molecules and integrins), cell adhesion (cadherins), signal transcription and transduction (Caro *et al.*, 2005).

Probiotics have also been found by several researchers to decrease fecal concentrations of enzymes (glycosidase, B-glucuronidase, azoreductase, and nitroreductase) and secondary bile salts and reduce the absorption of harmful mutagens that may contribute to colon carcinogenesis (Rafter, 1995). Normal intestinal flora can influence carcinogenesis by producing enzymes (glycosidase, B-glucuronidase, azoreductase, and nitroreductase) that transform precarcinogens into active carcinogens (Goldin, 1990; Pedrosa *et al.*, 1995). Lactobacillus *acidophilus* and *L. casei* supplementation in humans helped to decrease the levels of these enzymes (Lidbeck *et al.*, 1991). In mice, these bacterial enzymes were suppressed with the administration of *Lactobacillus GG* (Drisko *et al.*, 2003). Several mechanisms have been proposed as to how lactic acid bacteria may inhibit colon cancer, which includes enhancing the host's immune response, altering the metabolic activity of the intestinal microbial communities, binding and degrading carcinogens, producing antimutagenic compounds, and altering the physiochemical conditions in the colon (Hirayama & Rafter, 2000; Kumar *et al.*, 2011a, b). Oral administration of LAB has been shown to effectively reduce DNA damage, induced by chemical carcinogens, in gastric and colonic mucosa in rats (Li & Li,

2003). By comet assay, *L. acidophilus*, *Lactobacillus gasseri*, *Lactobacillus confusus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, and *B. longum* were antigenotoxic toward *N*'-nitro-*N*-nitrosoguanidine (MNNG; Pool-Zobel *et al.*, 1996). These bacteria were also protective toward 1, 2-dimethylhydrazine (DMH)-induced genotoxicity. Metabolically active *L. acidophilus* cells, as well as an acetone extract of the culture, prevented MNNG-induced DNA damage, while heat-treated *L. acidophilus* was not antigenotoxic. Azomethane-induced colon tumor development was also suppressed with a decrease in colonic mucosal cell proliferation and tumor ornithine decarboxylase and ras-p21 activities (Hirayama & Rafter, 2000). There was a report on the antitumorigenic activity of the prebiotic inulin, enriched with oligofructose, in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* in the azoxymethane (AOM)-induced colon carcinogenesis rat model (Femia *et al.*, 2002). Other lactic acid bacteria have also shown the ability to lower the risk of colon cancer; however, the relationship between enzyme activity and cancer risk needs further investigation.

Immunologic enhancement

There have been several reports indicating that lactobacilli used in dairy products can enhance the immune response of the host. Organisms that have been identified as having this property are *B. longum*, *L. acidophilus*, *L. casei* subsp. *rhamnosum*, and *Lactobacillus helveticus* (Isolauri, 2001). However, prospective probiotics should be tested in the future for the enhancement of the immunologic response. The measurements that should be considered are lymphocyte proliferation, interleukins 1, 2, and 6, TNF, prostaglandin E production, and serum total protein, albumin, globulin, and gamma interferon. The intrinsic properties of lactobacilli to modulate the immune system make them attractive for health applications. Enhanced phagocytic activity of granulocytes, cytokine excretion in lymphocytes, and increased immunoglobulin-secreting cells in blood are typical responses to probiotics, all of which are indicative of changes in the immune system. An inflammatory immune response produced cytokine-activated monocytes and macrophages, causing the release of cytotoxic molecules capable of lysing tumor cells *in vitro* (Philip & Epstein, 1986). The inflammatory cytokines IL-1 and TNF-α exerted cytotoxic and cytostatic effects on neoplastic cells in *in vitro* models (Raitano & Kore, 1993). Aatourri *et al.* (2002) observed increased lymphocyte proliferation in the spleen, peripheral blood, and Peyer's patches and also increased IFN-γ production in Peyer's patches and spleen of rats fed yogurt containing *L. bulgaricus* 100158 and *S. thermophilus* 001158. Because immune function declines with age, enhancing immunity in the elderly with probiotics would be of particular use (Gill & Rutherfurd, 2001). Regardless of the mechanisms involved, probiotics cultures have been shown to stimulate both nonspecific immunity and specific immunity. Possible stimulation of an immune response by probiotic bacteria may explain potential therapeutic and prophylactic applications of such cultures in the treatment for infections and carcinogenesis.

Enhancement of short-chain fatty acid production

Because the improved intestinal microbial communities with probiotics primarily involve the stimulation of intestinal fermentation, the stimulation of short-chain fatty acid (SCFA) production is one of the essential factors for the beneficial effects exerted by probiotics. A significant increase in indigenous lactobacilli in the large intestine as a result of probiotic *Lactobacillus* has been reported (Tsukahara & Ushida, 2001). Although increases in lactobacilli stimulate lactate production, lactate does not accumulate in the large intestine, except in those patients with short bowel syndrome and dyspeptic diarrhea (Tsukahara & Ushida, 2001). Rather, lactate is normally metabolized to acetate, propionate, or butyrate by lactate-utilizing bacteria (Bourriaud *et al.*, 2005; Belenguer *et al.*, 2006). Lactate-utilizing bacteria from the human flora have been previously identified as belonging to the Clostridia cluster XIVa, based on their 16S rRNA gene sequences (Duncan *et al.*, 2004). The increase in fecal SCFA by probiotic *Lactobacillus* would be due to this mechanism (Tsukahara *et al.*, 2006). In fact, the oral administration of the lactate-utilizing and butyrate-producing bacterium, *Megasphaera elsdenii*, with *Lactobacillus plantarum* has been shown to increase the butyrate production in the large intestine (Tsukuhara *et al.*, 2002). Thus, the administration of probiotics with other lactate-utilizing bacteria, butyrate-producing bacteria, in particular, could be a more effective way to achieve maximum health benefits.

Antiatherogenic and cholesterol-lowering attributes of probiotics

Coronary heart diseases and cardiovascular diseases (CVD), major causes of most death in adults, are conditions in which the main coronary arteries supplying the heart are no longer able to supply sufficient blood and oxygen to the heart muscle (myocardium). Although low-fat diets offer an effective means of reducing blood cholesterol concentrations, these appear to be less effective, largely due to poor compliance, attributed to low palatability and acceptability of these diets by the consumers. Therefore,

attempts have been made to identify other dietary components that can reduce blood cholesterol levels. Individuals with CVD and those with a higher risk of developing the condition are treated in a number of ways to help lower their LDL cholesterol and triacylglycerol (TAG) concentrations while elevating their high-density lipoprotein cholesterol. The role of fermented milk products as hypocholesterolemic agents in human nutrition is still equivocal, as the studies performed have been of varying quality, and statistically analysis with incomplete documentation being the major limitation of most studies. However, since 1974 when Mann & Spoerry (1974) showed an 18% fall in plasma cholesterol levels after feeding 4–5 liters of fermented milk per day for 3 weeks to Maasai warriors, there has been a considerable interest in the effect of probiotics on human lipid metabolism. Supplementation of diet with dairy products fermented with LAB has the potential to reduce serum cholesterol levels in humans and animals (Pulusoni & Rao, 1983). A significant decrease in serum cholesterol level in rats fed milk fermented with *L. acidophilus* has been reported (Grunewald, 1982). Mann (1977) showed that large dietary intake of yogurt lowered the cholesterolemia in humans.

Experiments by Gilliland *et al.* (1985) have shown that dietary elevation of plasma cholesterol levels can be prevented by the introduction of a *L. acidophilus* strain that is bile resistant and assimilates cholesterol. These findings were supported by Pereira & Gibson (2002) who demonstrated that probiotic strains were able to assimilate cholesterol in the presence of bile into their cellular membranes. Results, however, were influenced greatly by the bacterial growth stage, and inoculum using resting cells did not interact with cholesterol as also shown by studies conducted by Dambekodi & Gilliland (1998). St-Onge *et al.* (2000) extensively reviewed the existing studies from animal and human studies which detected that moderate cholesterol lowering was attributable to the consumption of fermented products containing probiotic bacteria. Studies by Gopal *et al.* (1996) also showed cholesterol removal by *Bifidobacterium* spp. and *L. acidophilus*. The possible mechanisms of action of probiotics are cholesterol assimilation by bacteria, deconjugation of bile salts, cholesterol binding to bacterial cell walls, and reduction in cholesterol biosynthesis (Pulusoni & Rao, 1983; Pereira & Gibson, 2002).

Probiotics in diabetes and obesity

The role of gut flora in the pathology of insulin resistance (type 2 diabetes) and obesity has been well documented by Ley *et al.* (2005). Animal and human studies have suggested that gut flora enhances the body weight gain and increases the insulin resistance, and these phenotypes are transmittable with gut flora during the implantation studies of microbiota from obese to normal and germ-free mice (Ley *et al.*, 2006; Turnbaugh *et al.*, 2006). The mechanisms associated with gut flora–mediated pathology of obesity and diabetes are through (1) increased energy harvest, (2) increased blood LPS levels (endotoxemia), and (3) low-grade inflammation (Delzenne *et al.*, 2011). Therefore, modulation of gut flora has been considered as a potential target to treat against obesity and diabetes. Probiotics are novel gut flora modulators, and their role in the prevention of and treatment for diabetes and obesity has been implicated in recent past by Yadav *et al.* (2007a, b, 2008). Yadav *et al.* (2007b, 2008) suggested that probiotic-supplemented fermented milk product called dahi (yogurt) dramatically suppressed diet-induced insulin resistance and protected from streptozotocin-induced diabetes in animal models. It was also observed that probiotic dahi suppressed the diabetes progression and its complication through enhancing antioxidant system (Yadav *et al.*, 2008). Though, the actual link between probiotic-mediated pathology of obesity and diabetes has been debated on the basis of farm animal's data (Raoult, 2008; Delzenne & Reid, 2009; Ehrlich, 2009). In relation to these controversies, *Bifidobacteria*, one of the important classes of probiotic organisms, have been found to be decreased in overweight women in comparison with normal weight women (Santacruz *et al.*, 2009). Recent studies have suggested that probiotic-based selective strains of *Lactobacilli* and *Bifidobacteria* show beneficial effects on obesity and type-2 diabetes (Aronsson *et al.*, 2010). Andreasen *et al.* (2010) reported that *L. acidophilus* decreased the insulin resistance and inflammatory markers in human subjects. More recently, Vajro *et al.* (2011) and others (Kang *et al.*, 2010; An *et al.*, 2011; Chen *et al.*, 2011; Naito *et al.*, 2011) showed that feeding of specific strains of *Lactobacilli* and *Bifidobacteria* ameliorate the progression of obesity and diabetes, suggesting that probiotic-mediated modulation of gut flora can be a potential therapy against obesity and diabetes. Although animal studies have shown promising results in probiotic-mediated suppression of obesity and diabetes, very few studies in humans showed the significant effects. Hence, it is required to conduct well-designed studies for examining the efficacy of probiotic-based formulation in the treatment for obesity and diabetes. Also, the mechanism (s) of action for probiotic-based formulation is not completely understood; therefore, future studies should also be focused on describing the probiotic action–targeted molecules and organs in physiologic models.

Other potential benefits

Certain functional foods containing probiotic provide preformed lactase to gut and allow better digestion of

lactose. The regulatory role of probiotics in allergic disease was demonstrated by a suppressive effect on lymphocytes' proliferation and interleukin-4 generation *in vitro* (Sutas *et al.*, 1996). Subsequently, the immune inflammatory responses to dietary antigens in allergic individuals were shown to be alleviated by probiotics, this being partly attributable to enhance the production of anti-inflammatory cytokines (Pessi *et al.*, 2000) and transferring growth factor-β (Haller *et al.*, 2000). Probiotic bacteria also possess prophylactic and therapeutic properties. Other potential benefits include protection against vaginal or urinary tract infections, reduction in ulcers and intestinal tract infections, increased nutritional value, maintenance of mucosal integrity, reduction in catabolic products eliminated by kidney and liver, stimulation of repair mechanism of cells, breaking down and rebuilding hormones, relieving anxiety and depression, formation, maintenance, or reconstruction of a well-balanced indigenous intestinal and/or respiratory microbial communities, inhibiting decalcification of the bones in elderly people, and synthesis of vitamins and predigestion of proteins.

Molecular characterization of probiotics marker genes and surface layer protein (SlpA)

In view of high stakes involved in the exploration of their commercial value, particularly in the booming functional/health food market, the correct identification of probiotic cultures has become extremely important to rule out the possibility of false claims and to resolve disputes concerning their identity in probiotic preparations (Mohania *et al.*, 2008). The phylogenetic information encoded by 16S rRNA gene has enabled the development of molecular biology techniques, which allow the characterization of the whole human gut microbiota (Lawson, 1999). These techniques have been used in monitoring the specific strains as they have high discriminating power. Numerous molecular techniques have been exploited for the identification of various putative probiotic marker genes such as bile salt hydrolase (BSH), mucus-binding protein (mub), fibronectin-binding protein (fbp) for the screening of probiotic strains.

Bile salt hydrolase (BSH) gene

BSH, an intracellular enzyme found commonly in certain intestinal bacteria, plays a vital role. BSH catalyzes the hydrolysis of glycine- or taurine-conjugated bile acids into the amino acid residue and deconjugated bile acid. The ability of probiotic strains to hydrolyze bile salts has often been included among the criteria for the selection of probiotic strain, and a number of BSHs have been identified and characterized. It has been investigated that *Lactobacillus* isolates of human origin along with *Bifidobacterium* also possess *bsh* homologs in their genome. Sequence analysis of these *bsh* homologs establishes intraspecies heterogeneity and interspecies homogeneity, which might be due to the horizontal transfer of *bsh* gene from one species to other. With the completion of some probiotic genome projects, analyses of sequenced probiotic (*Lactobacilli* and *Bifidobacteria*) strains reveal that many possess more than one *bsh* homolog and each BSH may respond to different types of bile or perhaps different length of exposure to bile. Therefore, BSH activity by a probiotic bacterium may be a desirable property because it could maximize its prospects of survival in hostile environment of GI tract and hence can be used as one of the potential markers for the screening of probiotic strains. Because large amounts of deconjugated bile salts may have undesirable effects for the human host, concerns may arise over the safety of administering a BSH-positive probiotic strain. However, the bacterial genera that would most likely to be used as probiotics (*Lactobacilli* and *Bifidobacteria*) are not capable of dehydroxylating deconjugated bile salts, and so the majority of the breakdown products of BSH activity by a probiotic strain may be precipitated and excreted in feces. Hence, the ability of probiotic strains to hydrolyze conjugated bile salts has often been included among the criteria for probiotic strain selection (FAO/WHO, 2002).

Mucus-binding protein (Mub), Fibronectin-binding protein (FbpA), and surface layer protein (SlpA)

Roos & Jonsson (2002) identify the *mub* gene encoding mucus-binding protein in *Lactobacillus reuteri* ATCC 53608 (strain 1023). Using the immunoglobulin G (IgG) fraction of an antiserum against cell surface proteins of *L. reuteri* ATCC 53608 (strain 1023), they screened a phage library and identified a number of clones that were reactive with the antiserum and adhered to mucus. Subcloning resulted in the identification of the *mub* gene, encoding a very large sortase-dependent protein (SDP) with a highly repetitive structure (3000 residues). Domains with the two main types of repeats, that is, Mub1 and Mub2, were shown to adhere to mucus after recombinant expression in *Escherchia coli*. In another *L. reuteri* strain, 100-23, a similar approach using an antiserum against the surface proteins was used to identify the *lsp* (large cell surface protein) gene, which encodes a high molecular mass cell wall protein, Lsp (Walter *et al.*, 2005). Mutational analysis showed a reduced ecological performance of the *lsp* mutant in the murine gastro intestinal tract (GIT). Boekhorst *et al.* (2005) performed

an in *silico* search for potential mucus-binding proteins present in several publicly available databases. They reported that a total of 48 proteins containing at least one MUB domain were identified in 10 lactic acid bacterial species. Callanan *et al.* (2008) reported that these mucus-binding proteins are involved mainly in GIT colonization as observed from the genome sequence of the dairy isolate *L. helveticus* DPC4571. A striking difference between the various mucus-binding proteins is the number of repeats of the MUB domain, and it might be interesting to investigate whether the number of repeats correlates with the capacity of binding to mucus (Boekhorst *et al.*, 2006).

Buck *et al.* (2005) reported the genes encoding FbpA, Mub, and SlpA all contribute to the ability of *L. acidophilus* NCFM to adhere to Caco-2 cells *in vitro*, confirming that adhesion is determined by multiple factors. *mub* and *fbpA* mutations resulted in 65% and 76% decreases in adherence, respectively. In a similar study, VanPijkeren *et al.* (2006) mined the genome of *L. salivarius* UCC118 for the presence of sortase gene homologs and genes encoding SDPs. The sortase gene *srtA* was deleted, three genes encoding SDPs (large surface protein *lspA*, *lspB*, and *lspD*) were disrupted, and the capacity of adherence of these mutants to HT-29 and Caco-2 cells was investigated. Both the *srtA* and the *lspA* mutant showed a significant decrease in adherence. While the adherence of the *srtA* mutant was on average 50% of wild-type levels, the *lspA* mutant adhered at around 65%, only slightly better than the Sortase *srtA* mutant, indicating that LspA plays a key role in adherence to these intestinal cells.

Mechanism of action of probiotics

Probiotic bacteria have multiple and diverse influences on the host. Different organisms can influence the intestinal luminal environment, epithelial and mucosal barrier function, and the mucosal immune system. The numerous cell types affected by probiotics involve epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells. There are significant differences between probiotic bacterial genera and species. These differences may be due to various mechanism of action of probiotics. It is crucial that each strain be tested on its own or in products designed for a specific function. Molecular research on these probiotics pays attention to these strain-specific properties. Different probiotic strains have been associated with different effects related to their specific capacities to express particular surface molecules or to secrete proteins and metabolites directly interacting with host cells.

The effectiveness of probiotics is related to their ability to survive in the acidic and alkaline environment of gut as well as their ability to adhere and colonize the colon. The mechanisms for the improved mucosal barrier are achieved by providing a means of limiting access, with respect to pH, redox potential, hydrogen sulfide production, and antimicrobial compounds/molecules, to enteric pathogens or by several interrelated system such as mucous secretion, chloride and water secretion, and binding together of epithelial cells. Hydrogen peroxide in combination with lactoperoxidase–thiocyanate milk system exerts a bactericidal effect on most pathogens (Kailasapathy & Chin, 2000). *Bacillus clausii* constitute < 1% of gut microbial communities, stimulate CD4 proliferation, and produce bacteriocins to limit the growth of potential pathogens. Microbial communities also enhance nutritive value by producing several enzymes for the fermentation of nondigestible dietary residue and endogenously secreted mucus (Roberfroid *et al.*, 1995) and help in recovering lost energy in form of short-chain fatty acids. They also have a role in the synthesis of vitamins (Conly *et al.*, 1994) and in the absorption of calcium, magnesium, and iron (Younes *et al.*, 2001). Some examples of host benefit and suspected mechanism have been summarized in Table 1.

Prospective applications of probiotics in developing healthful foods

A growing public awareness of diet-related health issues and mounting evidence regarding health benefits of probiotics have increased consumers demand for probiotic foods. A number of food products including yoghurt, frozen fermented dairy deserts, spray-dried milk powder, cheeses, ice cream, freeze-dried yoghurt (Nagpal *et al.*, 2007; Kumar *et al.*, 2009a; Nagpal & Kaur, 2011), and fruit juices (Nagpal *et al.*, 2012) have been suggested as delivery vehicles for probiotic to consumer. It has been suggested that approximately 10^9CFU per day of probiotic microorganisms is necessary to elicit health effects. Based on the daily consumption of 100 g or mL of probiotic food, it has been suggested that a product should contain at least 10^7 cells per g or mL of a food, a level that was also recommended in Japan (Ross *et al.*, 2002). The most popular food delivery systems for probiotic have been fermented milk and yoghurt. A few studies have shown that many commercial yoghurt products have failed to successfully deliver the required level of viable cells of probiotic bacteria (Dave & Shah, 1997). Cheeses have a number of advantages over fresh fermented products (such as yoghurt) as a delivery system for viable probiotic to GI tract. Cheeses tend to have a higher pH and more solid consistency where the matrix of the cheese and its relatively high fat content may offer protection to probiotic bacteria during passage through the GI tract. Cheese also has high buffering capacity than yoghurt (Gardiner *et al.*, 1998). Overall, the major points to be

addressed while incorporating probiotics into foods are the selection of a compatible probiotic strain/food type combination; using food processing conditions that are compatible with probiotic survival; ensuring that the food matrix supports probiotic growth (if fermentation is required); selecting a product matrix, packaging, and environmental conditions to ensure adequate probiotic survival over the product's supply chain and during shelf storage; and finally ensuring that addition of the probiotic does not adversely impact on the taste and texture of the product.

Carriers for probiotics

Probiotics are normally added to foods as a part of the fermentation process. The emphasis for prolonged survival of probiotics in the food matrix has resulted in the alteration in the functionality and efficacy of the food product. In order to exert health benefits, probiotic bacteria must remain viable in the food carriers and survive the harsh condition of GI tract, with a minimum count of 10^6 CFU g^{-1}. The nature of food carrier can affect the stability of the probiotic microorganisms during GI transit. Although dairy-based products are suggested to be the main carriers for the delivery of probiotics, other nondairy-based products such as soy and fruits can be exploited as a potential carrier of probiotic microorganisms because of the increasing demand for new flavor and taste among consumers. A brief idea about the variety of products that serve as carriers for probiotics is given in Table 4.

Legislation and safety regarding probiotics

The regulatory status of probiotics as a component in food has to be established on an international level. A regulatory framework should be established to better address probiotic issues, including efficacy, safety, labeling, fraud, and claims. Probiotic products shown to confer defined health benefits on the host should be permitted to describe these specific health benefits. Surveillance systems (trace-back, postmarketing) should be put in place to record and analyze adverse events associated with probiotics in food and monitor long-term health benefits. Probiotic products should be made more widely available, especially for relief work and to populations at high risk of morbidity and mortality. Foods that could be regarded as functional foods are subject to regulations drawn up for other food groups. The US Food and Drug Administration (FDA) defined four food categories: conventional foods, constituting the largest category and including articles of food and drink that do not fall into the other three categories such as foods for special dietary use; medical foods; and dietary supplements. According to Berner & O'Donnell (1998), it is possible to envision 'functional foods' in any of the categories of foods and supplements mentioned above. From a

Table 4. Details of the products that serve as carriers for probiotics

Carrier	Products	Probiotics	References
Dairy based	Sweet-acidophilus milk	*L. gasseri*	Usman & Hosono (1999)
	Ice cream	*L. johnsonii*	Alamprese *et al.* (2002)
	Whey drink	*L. casei*	Drgalić *et al.* (2005)
	Whey cheese	*B. animalis, L. acidophilus, L. brevi, L. paracasei*	Madudeira *et al.* (2005)
	Natural-set yogurt	*L. acidophilus, L. casei, Bifidobacterium*	Donkor *et al.* (2007)
	Low-fat cheddar cheese	*L. casei*	Sharp *et al.* (2008)
	Yogurt	*L. acidophilus, L. casei, B. bifidum*	Sendra *et al.* (2008)
Soy based	Soymilk	*Lactobacillus, Bifidobacterium, Streptococcus thermophilus*	Donkor *et al.* (2007)
	Soy cream cheese	*L. acidophilus*	Liong *et al.* (2009)
	Soymilk	*L. acidophilus, L. casei, Bifidobacterium*	Yeo & Liong (2010)
	Soymilk	*L. acidophilus., L. gasseri*	Ewe *et al.* (2010)
	Soymilk	*L. plantarum*	Bao *et al.* (2011)
Juice based	Tomato juices	*L. casei A4, L. delbrueckii D7*	Yoon *et al.* (2004)
	Cabbage juices	*L. plantarum, L. acidophilus*	Yoon *et al.* (2005)
	Beet juice	*L. plantarum, L. casei, L. delbrueckii*	Yoon *et al.* (2006)
	Orange and pineapple juice	*L. casei, L. rhamnosus GG, L. paracasei, L. acidophilus LA39*	Sheehan *et al.* (2007)
	Carrot juice	*B. lactis Bb-12, B. bifidum B7.1, B3.2*	Kun *et al.* (2008)
	Tomato, orange, and grape juice	*L. plantarum, L. acidophilus*	Nagpal *et al.* (2012)

legislative standpoint, probiotic-containing foods could fit into several of the four categories of foods described by the FDA; however, there is no explicit recognition of any health benefits of probiotic-, prebiotic-, or culture-added dairy foods in the United States.

Government regulations regarding safety assessment differ among countries, and the status of probiotics as a component in food is currently not established on an international basis. For the most part, probiotics come under food and dietary supplements because most are delivered by mouth as foods and, as such, are allowed to make only general health claims. The factors that must be addressed in the evaluation of safety of probiotics include pathogenicity, infectivity, and virulence factors comprising toxicity, metabolic activity, and the intrinsic properties of the microorganisms. Donohue & Salminen (1996) provided some methods for assessing the safety of lactic acid bacteria through the use of *in vitro* studies, animal studies, and human clinical studies and indicated that some current probiotic strains are reported to fulfill the required safety standards. Salminen & Marteau (1997) also proposed studies on intrinsic properties, pharmacokinetics, and interactions between the host and probiotics as means to assess the safety of probiotics. It was recognized that there is a need to accurately enumerate the probiotic bacteria in food products to include them on a label and that proper manufacture and handling procedures be employed to ensure the maintenance of viability and probiotic activity through processing, handling, and storage of probiotic foods, including powdered milk products. Good evidence exists that specific strains of probiotics are safe for human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated to other strains without experimentation. As there has been an increased influx of probiotic products in the Indian market during the last decade, therefore an initiative was taken by the Indian Council of Medical Research and Department of Biotechnology, Government of India, to formulate guidelines for the regulation of probiotic products in the country (Ganguly *et al.*, 2011), defining a set of parameters required for a product/strain to be termed as 'probiotic'. These include the identification of the strain, *in vitro* screening for probiotic characteristics, and *in vivo* animal and human studies to establish efficacy, requirements for labeling of the probiotic products with strain specification, viable numbers at the end of shelf-life, storage conditions, etc., so as to help the consumers to safeguard their awareness.

Validation of health claims

To validate or substantiate a health-related claim, the proposed relationship between the product and the health-related end point should be identified, and appropriate measurements of both should be indicated. The interests of patients and consumer involvement are becoming integral part of clinical development and should be taken into consideration. For regulatory purposes, health-related claims require sound evidence from all available sources. Positive evidence should not be outweighed by negative evidence, and sufficient evidence based on human experience should be available to support the safety and efficacy, including pre- and postmarketing experience. The greater the consistency of evidence from different sources, the stronger the evidence will be.

The Nutrition Labeling and Education Act of 1990 gives the US Food and Drug Administration (FDA) the authority to regulate health claims on food labels. These claims describe the link between specific nutrients or substances in food, and a particular disease or health-related condition. The process of reviewing the scientific evidence of health claims involves the following steps: define the substance–disease relationship that is the subject of the claim, identify relevant studies, classify the studies, rate the studies on the basis of quality, rate the studies on the basis of the strength of their body of evidence, and report the studies' rank order.

Future prospects: toward genetically modified designer probiotics

Genetic manipulation offers the potential to enhance the existing probiotic properties of an organism or to load an organism with probiotic properties (Steidler, 2003). Elucidation of mechanisms of activity of a probiotic could enable the manipulation of organisms to create specific and targeted probiotics. Although consumer resistance to genetically modified organisms is such that GMO probiotic foods are unlikely in the near future, potential clinical applications to ameliorate or prevent chronic intractable diseases may be more readily accepted. For instance, Steidler (2003) treated mice with genetically modified *Lactococcus lactis* to deliver mouse cytokine IL-10 at the intestinal mucosa to prevent colitis, demonstrating that probiotics can be designed to produce potent bioactive chemicals. Braat *et al.* (2006) also constructed a biologically contained *L. lactis* to produce human IL-10 and treated Crohn's disease patients with this GM *L. lactis* in a phase-1 placebo-uncontrolled trial. A decrease in disease activity was observed with minor adverse effects, and containment of the organism was achieved through its dependency on thymidine for growth and IL-10 production.

Synbiotics

Another possibility of gut microbial community management is the use of synbiotics, where probiotics and prebiotics

are used in combination. A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the host health (Gibson & Roberfroid, 1995). The combination of suitable probiotics and prebiotics enhances the survival and activity of the organism. The combination of prebiotic and probiotic has synergistic effects because in addition to promoting the growth of existing strains of beneficial bacteria in the colon, synbiotics also act to improve the survival, implantation, and growth of newly added probiotic strains. The synbiotic concept has been widely used by European dairy drink and yoghurt manufacturers such as Aktifit (Emmi, Switzerland), Proghurt (Ja Naturlich Naturprodukte, Austria), Vifit (Belgium, UK), and Fysiq (the Netherlands; Niness, 1999). The combination of *Bifidobacterium* and oligofructose was reported to synergistically improve colon carcinogenesis in rats compared to when both were given individually (Gallaher & Khil, 1999). Another study reported that a synbiotic containing *Pediococcus pentoseceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei*, and *L. plantarum* with four fermentable fibers namely β-glucan, inulin, pectin, and resistant starch reduced the occurrence of post-operation infections from 48% to 13% in 66 liver transplant patients (Rayes *et al.*, 2005). Most of the claims on benefits of different synbiotics are on general health (Gibson & Roberfroid, 1995). There have yet been any clinical trials on suitable combinations of synbiotics that specifically target reduction in serum cholesterol level in animals and humans. *Bifidobacteria* and *Lactobacilli* are the most frequent target organisms for prebiotics. Although there is growing interesting development of new functional foods with synbiotics, the concept of synbiotics has been studied to a limited extent and needs further investigations. Only a few human studies have been carried out on the effectiveness of synbiotics (Morelli *et al.*, 2003).

Conclusion

There are evidences from well-conducted clinical trials of beneficial health effects from probiotics in a range of clinical conditions. The concept of 'synbiotics' has recently been proposed to characterize health-enhancing food and supplements used as functional food ingredients in humans, and with the advent of the functional food concept, it is clear that there is an important niche for these probiotic-based approaches. Although from the ongoing research, more of promising potential health effects of probiotics are being observed, more standardized and verifiable clinical studies are needed to demonstrate the safety, efficacy, and limitations of a putative probiotic, to determine effects on the immune system in healthy and diseased individuals and effects of long-term consumption, and to resolve whether it is superior to existing therapies. Also, the prospect of GM probiotics targeted for clinical conditions demands a rigorous safety strategy to prevent spread into the environment and dissemination of the genetic modification.

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