REVIEW

Probiotics and Prebiotics

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ABSTRACT

Cereal Chem. 80(2):113–117

Probiotics, bacteria from the genera *Bifidobacterium* and *Lactobacillus*, and yeast, *Saccharomyces*, as well as prebiotics belonging to the group of dietary fiber (inulin with low degree of polymerization, fructose-derived oligosaccharides, and resistant starch) are natural factors useful in prophylaxis and therapy of several common diseases including some types of cancer. They are available commercially and can be introduced to produce so-called functional food. Probiotics and prebiotics can be utilized either separately or jointly (as synbiotics or eubiotics). Mechanisms of both biotics are discussed. The role of cereals in probiosis is considered. Possibilities for extension of the uses of the original Chinese probiotic, *chaw tofu*, are also considered.

For millennia, humans have utilized microorganisms in processing foodstuffs such as in milk fermentation and the production of alcoholic and nonalcoholic beverages. As early as the Greco-Latin civilization, and particularly in the Far East, dietary composition and nutrition style was considered an essential component of natural medicine. Benefits from consumption of buttermilk and yogurt have been known for centuries, but only in the 20th Century have the positive results from the consumption of fermented dairy products been related to probiotics present in these products. Probiotics and the phenomenon of probiosis were discovered by Mietchnikoff (1908). Probiosis can be defined as the positive effect of consumption of fermented dairy products with cultures of lactic acid bacteria on the equilibrium of intestinal microflora. In earlier years, it did not develop into any clinical application. Priority was given to newly discovered antibiotics such as sulfonamides. Recently, side effects of therapy with antibiotics and development of bacteria resistant to them have provoked increasing interest in probiosis. Benefits from the use of probiotics were documented and the positive effects of "good bacteria" from nutrients present in the human diet were recognized. The term functional food describes foodstuffs which, apart from their nutritive value, evidently improve health and well-being of consumers. They may be applied in prophylaxis and therapy of certain diseases and for promotion of health. Functional foods can contain probiotics or prebiotics. Such functional foods should be part of the human diet from early childhood. The Committee on Nutrition of the American Academy of Pediatrics (1992) documented that cow's milk does not cover the nutritional demands of children 1–3 years old. Therefore, modified milk is recommended for them (ESPGHAN 1990).

Probiotics: Definition and Mechanism of Action

Probiotics are defined as living microorganisms in foodstuffs which, when taken at certain levels in nutrition, provide equilibration of the intestinal flora, and hence have a positive effect on the health of the consumer (Table I). Probiotics are selected from the strains most beneficial for the host intestinal bacteria, that is bacteria from the genera *Bifidobacterium*, *Lactobacillus*, and yeast.

Among criteria for microorganisms to be included in the probiotics group are 1) survival on passing through gastrointestinal tract at low pH and on contact with bile; 2) adhesion to intestinal epithelial cells; 3) stabilization of the intestinal microflora; 4) nonpathogenicity; 5) survival in foodstuffs and possibility for production of pharmacopoeia lyophilized preparations; 6) fast multiplication, with either permanent or temporary colonization of the gastrointestinal tract; and 7) generic specificity of probiotics.

In addition to lactic acid bacteria, *Saccharomyces boulardii* is used as a probiotic. This species of yeast was isolated from litchi fruits in Indonesia (McFarland et al 1993). Its resistance to all but mycocidal antibiotics is a considerable advantage over bacterial probiotics (Boddy et al 1991), although resistance to antibiotics is not a general probiotic property.

Greater resistance of organisms to intestinal infections due to inhibition of the growth of pathogenic bacteria (Gibson and Saavedra 1997) is another benefit from the use of probiotics. Probiotics fill an ecological niche by adhering to intestinal walls (Coconnier et al 1997). They also actively affect the growth of other bacteria because they decrease pH levels and, under specific conditions, they can produce hydrogen peroxide and bacteriocins (nisin and pediocin) (Saavedra 1995). They competitively utilize nutrients such as simple carbohydrates necessary for development of *Clostridium difficille*. Specific immuno mechanisms can be mobilized. Probiotics containing, for example, *Bifidobacterium bifidum* and *Streptococcus thermophilus* were administered to children with rotaviral diarrhea, resulting in faster seroconversion within IgA and IgM antibodies accompanied by the growth of cells producing IgM antibodies (Isolauri et al 1991; Saavedra et al 1994, 1998). Intensification of the production of sIgA antibodies induced by probiotics in the gastrointestinal tract has been described (Buts et al 1990).

The positive effect of probiotics is not limited solely to the gastrointestinal tract. Enzymatic hydrolysis with participation of bacteria increases bioaccessibility of lipids and proteins and reduces allergenicity of foodstuffs (Friend and Shahani 1984; Sutas et al 1996). Children receiving mixtures enriched in probiotics exhibited significantly fewer allergy symptoms than the control group (Majamaa and Isolauri 1997). Probiotics can digest lactose, and symptoms of lactose nontolerance diminish as the lactose level in fermented dairy products decreases (Kim and Gilliland 1983). Probiotics are beneficial for people with primary and secondary shortages of endogenic lactase (Marteau et al 1990). Probiotics synthesize B-group and K vitamins as well as cytoprotective short-chain fatty acids and polyamines (putrescine, spermine, and spermidine) (Buts et al 1994). Lactic acid bacteria increase the level of the B-group vitamins, and bacteria in yogurt increase the level of folic acid, niacin, and riboflavin up to 20-fold (Shahani and Chandan 1979; Deeth and Tomine 1981). The intestinal microflora play a key role in circulating estrogens in women, mobilizing bound estrogen, equivalent to reduction of excessive sex hormones (Goldin et al 1982; Gorbach 1984).

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Publication no. C-2003-0210-01R.

TABLE I
Microorganisms Used as Probiotics for Humans

Probiotic (bacteria or yeast)	Food Product	Therapeutic Agent Registered
Lactobacillus acidophilus	Sour milk, yogurt, buttermilk, fermented cheese	*L. acidophilus*, Lacidofil, Lakcid, Lakcid forte, Trilac, Yogurt
L. casei Shirota strain	Cheese, Yakult	
L. delbrueckii ssp. *bulgaricus*	Yoghurt	Trilac
L. plantarum	Cream, fermented bread	
L. rhamnosus	. . .[a]	*L. acidophilus*, Lacidofil
L. reuteri	. . .	
Bifidobacterium adolescenti	. . .	
B. bifidum	Selected dairy products	Trilac, Lactobif
B. Breve, B. Infantis, B. Longum, B. lactis	Infant's milk and probiotic Cheddar-like cheese	Junior Bifidus Nestle
Saccharomyces boulardii, Streptococcus thermophilus	. . .	Enterol

[a] Probiotics isolated from various sources. They are commercially available and used in foods.

Bacteria also metabolize some drugs, for instance anthraquinone glycosides; this activity may be considered as an unwanted effect, or in some cases it may reduce an overdosage effect. Preliminary reports from experiments with animals indicate a decrease in the frequency of colon cancer under the influence of probiotics. It is a result of excretion from the colon of bacterially produced ammonia, aliphatic amines, indols, phenols, and sulfur compounds. In this manner, concentration of these carcinogens is reduced (Reddy 1998; Pierre et al 1999). In addition, activity of other enzymes participating in carcinogenesis, such as azoreductases and nitroreductases, is reduced.

Decrease in the tension of intestinal walls caused by bacterial fermentation leading to the production of hydrogen, carbon dioxide, hydrogen sulfide, and methane is a less essential beneficial phenomenon. Links between probiotic uptake and insulin-independent diabetes and obesity are under consideration (Seewi 1999). For cats, supplementation of animal fodder with lactosucrose reduces odor of excrement (Hussein et al 1999).

Lactobacillus plantarum increases the level of ω-3 unsaturated fatty acids in foodstuffs (Bengmark 1998). Probiotics protect foodstuffs from multiplication of harmful, potentially pathogenic, bacteria. This may be a reason for faster increase in body mass among laboratory animals fed with fodder modified with probiotics, for instance, yogurt containing living bacterial cells (Hargrove and Alford 1980). Similar results were found in experiments with infants fed with mixtures rich in *L. acidophilus*. Their body weight increased faster than that of infants fed with mixtures free of probiotics (Robinson and Thompson 1952).

Prebiotics: Definition and Mechanism of Action

Prebiotics are defined as nondigestible substances (dietary fiber) that exert some biological effect on humans by selective stimulation of growth or bioactivity of beneficial microorganisms either present or therapeutically introduced to the intestine. Prebiotics undergo fermentation by beneficial microflora in the large intestine. The microflora concerned are the organisms mentioned above as probiotics. Prebiotics are sources of energy for the microflora. Several nonstarchy polysaccharides such as pectins, guar gum, oat gum, oligosaccharides, sugar alcohols, and endogenic carbohydrates such as mucin and chondroitin sulfate usually do not metabolize in the stomach and pass to the intestine, nor are they digested by the intestinal microflora. Therefore, they cannot be considered prebiotics. Recently, Klahorst (2000) included polyols such as lactitol, mannitol, sorbitol, and xylitol to the prebiotics group.

Thus far, inulin with a low degree of polymerization oligofructosanes, and resistant starch comprise the prebiotics group, although further studies can, potentially, add some other poly- and oligo-saccharides to this group. Currently, particular attention is paid to saccharides in the inulin group (i.e., Glc*p*Fru*f* [α-D-glucopyranosyl-(β-D-fructofuranosyl)$_n$-1-D-fructofuranose] and Fru*p*Fru*f* [β-D-fructopyranosyl-(β-D-fructofuranosyl)$_n$-1-D-fructofuranose] where n = 10–60 (De Leenheer 1996) and fructooligosaccharides (FOS) apart from oligofructose with n = 2–9, and, eventually with D-xylose, D-galactose, D-glucose, and mannose residues. These compounds are also known as nondigestible oligosaccharides (NDO) (Van Loo et al 1999). Oligofructoses can be found in wheat, onion, garlic, endive, leek, asparagus, Jerusalem artichoke, and bananas. Mothers' milk contains relatively high levels (3–6 g/L) of oligosaccharides. FOS are selectively digested by bifidobacteria and stimulate growth of their colonies. Therefore, in the gut of infants fed with mothers' milk, populations of bifidobacteria are 10× higher than in those of infants fed infant formula (Kunz and Rudloff 1993).

Some published results indicate that, in addition to stimulation and activation of useful microorganisms, products of bacterial fermentation of prebiotics are also beneficial to humans. FOS influence homeostasis of cells in the intestinal walls and hinder them. Immunomodulation and bacteriostatic activity might result from blocking receptors open for interaction with pathogenic bacteria. Oligosaccharides with mannose side chains obstruct adhesion of *Escherichia coli* to the intestinal walls (Zopf and Roth 1996). On the other hand, cellobiose, the plant disaccharide, reduces infectivity of *Listeria monocytogenes*. In experiments, adding FOS to animal fodder significantly decreased frequency of colon cancer in those animals. Recommended daily uptake of FOS for humans is 4 g (Gibson 1998). Experiments with animals revealed that diets containing either inulin or resistant starch (5–20%) improves uptake of calcium, iron, and zinc (Delzenne et al 1995; Younes et al 1996). Inulin stimulates absorption of calcium in humans without any such effect on zinc, iron, and magnesium, but the doses of inulin used were much lower (15–40 g/day) (Coudray et al 1997). This observation can be described in terms of osmotic action of oligosaccharides, acidification of intestine content by bacterial fermentation, and formation of readily soluble magnesium and calcium salts of lower fatty acids generated on fermentation. It could also be related to secondary hypertrophia of the mucous membrane of the colon.

Rats supplied with inulin at a level of 10% in the diet showed a decrease in the level of triglycerides and cholesterol void and after a meal (Delzenne Robertfroid 1994; Fiordaliso et al 1995). However, relevant data in healthy people are equivocal. At a daily uptake of ≈10 g of inulin either decreases in triglycerides and cholesterol (Canzi et al 1995) or no changes in blood lipid level were observed (Pedersen et al 1997). Significant decreases in total cholesterol and LDL cholesterol were observed in patients with hypercholesterolemia at an inulin supply of up to 18 g/day (Davidson et al 1998). Reasons for declines in level of triglycerides and cholesterol in blood plasma remain unclear. Decrease in VLDL fraction in rats after administration of inulin suggests inhibition of liver lipogenesis (Fiordaliso 1995). Further studies revealed a 40% decrease in synthesis of triglycerides in isolated hepatocytes of rats that took inulin before the test (Fiordaliso 1995). This might result from a decrease in the activity of lipogenic enzymes of the liver. In rats, inulin also inhibited production of carcinogenic sialomucins and stimulated synthesis of anticarcenogenic sulfomucins (Cassidy et al 1990; Fontaine et al 1996). Inulin improves colonic action because facilitation of defecation is achieved by osmotic increase in the volume of stool (Jenkins et al 1999). Perhaps, the positive effects of supplementation of food with inulin should be considered as the result of bacterial fermentation of inulin. It is a good example of cooperative action of pre- and probiotics.

Synbiotics (Eubiotics)

Probiotics and prebiotics simultaneously present in a product are called either synbiotics or eubiotics. Such a combination aids survival of the administered probiotic and facilitates its inoculation into the colon. Additionally, the prebiotic induces growth and increases activity of positive endogenic intestinal flora. Experiments with rats showed that synbiotics protect the organism from carcinogens significantly better than do either prebiotics or probiotics separately (Gallaher and Gill 1999).

Functional Food and Probiosis

The food industry makes wide marketing use the concept of functional food. Functional food may be considered as a therapeutic aid available without prescription. With functional food, various functions of organisms can be modulated. Functional food not only prevents various diseases but also protects from effects of environmental pollution. By 1997, 90% of consumers were convinced that even slight modulation of their diet could improve their health. The market responded quickly to this opinion. Sales of functional foods increased by 15% annually. In 1997, European sales of yogurt brought a profit of $2 billion to manufacturers (Young 1998). Yogurt is a classic example of a functional food with probiotics. Yogurt with probiotic, called bio-yogurt, should contain living bacterial cultures. Despite the great popularity of functional foods, regulations are lacking for describing it as bio-food (i.e., containing probiotics). In 1997, only California and Oregon in the United States had introduced regulations for dairy products (Sanders 1998). According to these regulations, bio-dairy products should contain 2 million bacteria in 1 cm^3 at the end of the recommended storage period. The daily dose of probiotic organisms should reach 1 billion cells. The titer of bacteria in fermented drinks reaches 10^8–10^9/cm^3 and decreases with storage.

Several foodstuffs with probiotics and prebiotics are available in the European, American, and Asian marketplace. Usually dairy products from fresh milk (e.g., sweet *Acidophilus* in the United States), kefirs, and yogurts are those containing probiotics. Also, fruit juices with *Lactobacillus* and bifidobacteria and vitamins, (Plus3 juice in the United States) are available. Moreover, there are tablets, capsules, and powders to use as additives to foodstuffs that contain lyophilized cultures of bacteria. Lactic acid bacteria are available as pharmacopoeia preparations such as lakcid, trilac, lacidofil, and entrol 250. Synbiotics are also available. Symbalance from Swiss producer Tonilait is a yogurt with *Lactobacillus reuterii, L. acidophillus, L. casei,* and inulin from endive. Probiotic plus oligofructose from German producer Bauer is a yogurt with *L. acidophilus* and *Bifidobacterium bifidum*, and inulin from endive. Yakult is a nonalcoholic beverage of sour taste containing *L. casei.* It has been made in The Netherlands since 1995 by a Japanese company according to a Japanese patent from the 1930s. It is available in Benelux, Germany, and United Kingdom. Pro Viva is a yogurt containing probiotics and oat flour. Danio is a yogurt supplemented with whole wheat grains. Junior Bifidus Nestle is probiotic milk for children. Recently, a probiotic cheddar-like cheese with *Bifidobacterium infantis* was developed (Daigle et al 1999).

There are novel technologies for commercial manufacture of FOS. One that is particularly cheap uses production from sucrose with fructosyltransferase (Grizard et al 1998). Possible modification of prebiotics with microelements could be a novel way toward enhanced bioavailability of macro- and microelements. Increased uptake of calcium aided by pro- and prebiotics could be utilized in therapy and prophylaxis of osteoporosis. Reduction of cholesterol in hypercholesterolemic patients with pro- and prebiotics is a promising approach to the prophylaxis of arteriosclerosis and heart ischemia. Studies are underway (Gibson 1998) on the role of probiotics in eradication of *Helicobacter pylori*, the bacterium responsible for stomach and duodenal ulcers.

Fermented vegetables are common components of oriental cuisine. Soybean with whole nonpeeled grains, *koji* containing *Aspergillus oryzae*, and sea salt (*miso*) used to be fermented by *Bacillus natto*, which might be considered the original oriental probiotic strain (Chaitow and Trenev 1990; Rimbaut 1998). Oriental cuisine, and primarily Taiwanese cuisine, has *chaw tofu* (stinky tofu), a traditional soybean curd product fermented in brine. The “stinky” character of this product comes from several days contact of the tofu with fermented vegetables and shrimps. *Bacillus sphaericus* and *Lactobacillus* species are the dominant bacteria responsible for fermentation (Lee et al 1999). Therefore, stinky tofu might be considered as a potential probiotic food, more likely to satisfy culinary habits of Asian populations than rather unpopular fermented dairy products.

Cereals as Potential Prebiotics

Traditionally grains, particularly whole grains, are used in nutrition as carriers of macro- and microelements, proteins, fiber, and vitamins. In the past 30 years, numerous studies have documented a beneficial role in controlling blood pressure, arterial health, and insulin levels. Their proper use may also facilitate weight loss. All these properties have been utilized for several purposes. For example, whole grain wheat constituted an essential part of the macrobiotic diet in Japan after the nuclear bomb detonations over Hiroshima and Nagasaki (Ishimaru et al 1971; Akizuki 1978) and continue to the present (Heidery 1992; Ferre 1997). A proposal for modern food capable of decontaminating radioisotopes in human organisms also includes whole grain wheat (Aleksandrowicz and Tomasik 1985). Even modified cereal products such as cereal flakes (Kuntz 2000), as well as highly processed cereal derivatives such as the oat-based fat replacer Oatrim (Inglett 1991, 1992; Inglett et al 1996) were not considered prebiotics. Until recently, they have been recommended as healthy foods for their strong hypocholesterolemic properties. Oatrim became an example of the tendency toward combining the activity typical for dietary fibers with prebiotic activity. Thus, nutritional value of this product was fortified by blending with amylose-rich additives and β-glucans (Li et al 2000). Also available on the market are preparations composed of prebiotics encapsulated in a polysaccharide matrix (Klahorst 2000). Polysaccharide components of cereals, which could potentially act as prebiotics, were supposed to be too readily digested before they reached the intestine.

Counting resistant starch and sugar alcohols in the group of prebiotics reflects the current tendency toward joint consideration of the physiological activity of fiber and prebiotics. The most attention has been paid to resistant starch (RS). RS in the large bowel positively influences the rate of fermentation of prebiotics by the colonic microflora (Cassidy et al 1994). RS itself is fermented by microflora into lower fatty acids (Phillips et al 1995; Noakes et al 1996; Topping et al 1997). This action is accompanied by removal of secondary bile acids (Phillips et al 1995; Topping et al 1997). Thus, food containing prebiotics and fiber were put into a group of products called nutraceuticals. Finnish scientists have identified some common foods as valuable nutraceuticals. Thus, Grasten et al (2000) have paid attention to rye bread, which improves bowel function and decreases the concentration of some compounds that are putative colon cancer risk factors in middle-aged women and men. Evidence was also presented that such products did not eliminate probiotic bacteria from the intestine and colon because probiotic microorganisms did not adhere to fecal mucus (Ouwerhand et al 2000).

The majority of cereal grains appeared to be too readily digested to play an effective role as prebiotics, or even as nutraceuticals. Therefore, designing genetically modified, less digestible cereals suitable as prebiotics was suggested (Gibson and Roberfroid 1995). High-amylose corn starch is one such possibility, and its physiological properties were recognized almost a decade ago (Brown 1994).

Resistant starches may be divided into four groups: 1) seed starch, being physically inaccessible, forms the first group (RS1); 2) granular starch, particularly high-amylose and banana starches resistance of which depends, among others, on tendency to swelling, forms the second group (RS2); 3) highly retrograded starches, usually produced either by extrusion cooking or by liberation from cereals by enzymatic digestion, constitute the third group (RS3); 4) chemically modified starches constitute the fourth, very promising group of resistant starches. The latter continues to evoke considerable interest among researchers. Recently, Teixiera et al (1999) found that RS comprises two fractions. One is resistant to hydrolysis with α-amylase and is a true RS and the other, so-called slowly digestible starch (SDS), undergoes slow hydrolysis by α-amylase. This observation opens a path to further study on milder (possibly physical) methods of starch modification leading to SDS. It is likely that at least partial removal of the amorphous content of the interior of starch granules could provide SDS. Studies by Szymonska et al (2000) showed that deep-freezing and thawing of potato starch resulted in removal of amylopectin to the granule surface. Prolonged soaking of starch in water, particularly potato starch, causes migration of amorphous amylopectin to the solution without cracking of granules (Korus and Tomasik 2001). It might be likely that starches so modified could be digested as SDS. But, thus far, relevant studies have not been conducted.

Precautions in Application of Probiosis

Few reports have appeared in the literature about side effects of probiotics. In some cases, pneumonia and endocarditis were reported under the influence of *Lactobacillus* bacteria as well as nonsymptomatic fungal infection caused by *Saccharomyces boulardii* (Zunic et al 1991; Rogasi et al 1998). Certain medicines may interact with probiotics. On occasion, some infections were noted that could be linked to probiotics. These examples were found among consumers with compromised immune systems.

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[Received March 13, 2001. Accepted November 15, 2001.]