Probiotics in human health: A current assessment

V. C. Suvarna and V. U. Boby*

Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. This has led to the coining of the term probiotics. Today probiotics are available in a variety of food products and supplements, and have got wide applications in the control of cholestrol, cancers, allergies, etc. This article discusses the summary of research on the health benefits of probiotics and offers practical information to help health professionals and even the layman.

'LET food be thy medicine and medicine be thy food', the age-old quote by Hippocrates, is certainly the tenet of today. With the growing interest in self-care and integrative medicine coupled with our health-embracing baby boomer population, recognition of the link between diet and health has never been stronger. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods, is the small but rapidly expanding arena of probiotics – live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance.

The first recorded probiotic was fermented milk for human consumption. After that, probiotics became popular with animal nutrition. The role of fermented milk in human diet was known even in Vedic times. But, the scientific interest in this area boosted after the publication of the book entitled *The Prolongation of Life* by Ellie Metchinkoff[1] in 1908. He suggested that people should consume fermented milk containing lactobacilli to prolong their lives. Accelerated aging is because of autointoxication (chronic toxemia), which is due to the toxins produced by gut microflora. Bulgarian peasants who were subjected to the experiments on longevity had consumed large quantities of sour milk. The pathological reaction might be removed and life expectancy could be enhanced by implanting lactic acid bacteria from Bulgarian yogurt[1]. Since then, researchers started investigations relating to the role of lactic acid bacteria in human and animal health.

Probiotics have been used as growth promoters[2], for lactose intolerance[3], antitumour and anticholestrolaemic effects[4,5]. Probiotics have been extensively studied under *in vitro* and *in vivo* conditions. The main fields of research with respect to probiotics are heart diseases, allergic reaction, cancer, diarrhoea, etc. The use of probiotics resulting in alleviation of lactose intolerance due to increased concentration of β-galactosidase in the small intestine[3], relief from constipation by increased bowel function[6], antitumour activities due to inhibition of tumour cells, destruction of carcinogens[7,8], etc. have been well documented. Intestinal infections caused by *Escherichia coli*, *Campylobacter fetus* subsp. *jejuni*, *Clostridium perfringens* and *C. botulinum* were reduced in the presence of *Lactobacillus* supplements[9]. The *Lactobacillus* has shown promising results and *Bifidobacterium longum* has been successfully used to reduce the after-effects of antibiotic therapy[10].

Features of probiotics

A good probiotic agent needs to be non-pathogenic, non-toxic, resistant to gastric acid, adhere to gut epithelial tissue and produce antibacterial substances. It should persist, albeit for short periods in the gastro-intestinal tract influencing metabolic activities like cholesterol assimilation, lactose activity and vitamin production.

The survival of probiotic organisms in the gut depends on the colonization factors that they possess, organelles which enable them to resist the antibacterial mechanisms that operate in the gut. In addition to the antibacterial mechanisms, they need to avoid the effects of peristalsis, which tend to flush out bacteria with food. This can be achieved either by immobilizing themselves or by growing at a much faster rate than the rate of removal by peristalsis. The probiotic strain needs to be resistant to the bile acid, e.g. *Bifidobacteria* strains proved significantly less acid-resistant than the *Lactobacillus* strains, when exposed to human gastric juice[11].

Composition of probiotics

Probiotics can be bacteria, moulds, yeast. But most probiotics are bacteria. Among bacteria, lactic acid bacteria are more popular. *Lactobacillus acidophilus*, *L. casei*, *L. lactis*, *L. helviticus*, *L. salivarius*, *L. plantrum*, *L. bulgaricus*, *L. rhamnosus*, *L. johnsonii*, *L. reuteri*, *L. fermentum*, *L. del-*

The authors are in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore 560 065, India.
*For correspondence. (e-mail: bobyunni@rediffmail.com)

brueckii, *Streptococcus thermophilus*, *Enterococcus faecium*, *E. faecalis*, *Bifidobacterium bifidum*, *B. breve*, *B. longum* and *Saccharomyces boulardii* are commonly used bacterial probiotics (Table 1).

A probiotic may be made out of a single bacterial strain or it may be a consortium as well[12]. Probiotics can be in powder form, liquid form, gel, paste, granules or available in the form of capsules, sachets, etc.

Cholesterol assimilation

Probiotic strains, especially lactic acid bacteria have a major role to play in the cholesterol lowering mechanism. As the cholesterol level keeps increasing in the serum, it leads to cardiac diseases. These cholesterol levels can be brought down using probiotics[9]. The mechanisms can be direct or indirect. Direct mechanism is either inhibiting the *de novo* synthesis or by decreasing the intestinal absorption of dietary cholesterol. Inhibition of *de novo* synthesis can be attained by hypocholesterolemic factors like lactose, calcium hydroxyl methyl glutarate, uric acid, orotic acid, whey proteins, etc. The dietary cholesterol absorption is reduced by three ways – assimilating, binding or by degradation. Probiotic strains assimilate the cholesterol for their own metabolism. Probiotic strains can get bound to the cholesterol molecule, and they are capable of degrading cholesterol to its catabolic products. The cholesterol level can be reduced indirectly by deconjugating the cholesterol to bile acids, thereby reducing the total body pool. The bile acids commonly occur in the form of bile salts with glycine and taurine.

Deconjugation by different lactic acid bacterial cultures was also tried using two forms of bile salts, viz. taurocholates and glycocholates. Most of the strains could deconjugate glycocholates. For example, out of the thirteen strains of *L. casei* tested, nine could deconjugate glycocholate whereas none of them was able to deconjugate taurocholate[13]. Maximum quantity of free bile acids was present with reduced (added with thioglycolate) medium, whereas it was minimum in the case of aerobic incubation with optimum pH of 6.0.

Grunewald[14] conducted an experiment, where rats were fed with fermented milk to study the growth response and lipid profiles. Rats were divided into three groups, viz. water (control); water + 10% milk, and water + 10% fermented milk. Fermented milk was prepared with probiotic strain *L. acidophilus*. Though the results were non-significant, there was a drastic reduction in serum cholesterol of fermented milk-fed rats, indicating that cholesterol levels in serum can be reduced by consumption of probiotics.

Gillilland *et al.*[4] also conducted an experiment on pigs which were fed with high cholesterol diet followed by feeding with probiotic strains of *L. acidophilus* P-47 and RP-32. Pigs were fed with high cholesterol diet for ten days and observations were made on 0, 5 and 10th day. From the 5th day onwards, Gilliland *et al.* found significant difference in treatments and also that *L. acidophilus* RP-32 performed better than *L. acidophilus* P-47 (Table 2).

The potential hypocholesterolemic effect of probiotics sparked much interest based on evidence from animal work and from human studies, when 0.5–5 l yogurt/day was consumed. However, the ingestion of realistic quantities of yogurt or probiotics in man has not been shown to reduce cholesterol levels significantly[15,16].

Tahri *et al.*[17] studied the removal of cholesterol by *B. longum*, *B. infantis*, *B. breve*, *B. animalis* and *B. thermophyllum* in the presence of bile salts and observed that the removal of cholesterol from the growth medium by *Bifidobacteria* strains is due to both bacterial assimilation and precipitation of cholesterol. Lin and Chen[18] investigated cholesterol-reducing abilities of six strains of *L. acidophilus* and found that *in vivo* hypocholesterolaemic ability is likely due to the assimilation of cholesterol by *L. acidophilus* cells or/and attachment of cholesterol to the surface of *L. acidophilus* cells.

Table 1. Commercially used probiotics Lactobacilli and Bifidobacteria

Strain	Country	Company
Lactobacillus rhamnosus GG	Finland	Valio Dairy, Helsinki
Lactobacillus johnsonii Lal	Switzerland	Nestle, Lausanne
Lactobacillus casei Shirota	Japan	Yakult, Tokyo
Lactobacillus acidophilus NCFM	USA	Rhodia, Madison
L. casei CRL-43i Gilliland (La-Mo)	USA	Chr. Hansens, Wisconsin
Lactobacillus reuteri SD 2112	USA	BioGaia, North Carolina
Lactobacillus plantarum 299V	Sweden	Probi, Lund
L. rhamnosus 271		
L. casei DN 014001	France	Danone
Lactobacillus delbruekii subsp *bulgaricus* 2038		
Streptococcus thermophilus 1131	Japan	Meiji milk products, Tokyo
Lactobacillus acidophilus SBT-2062		
Bifidobacterium longum SBT-2928	Japan	Snow brand milk products, Tokyo
Saccharomyces boulardii	USA	Biocodex, Seattle
B. longum BB536	Japan	Morinaga milk industry
Bifidobacterium breve Yakult	Japan	Yakult, Tokyo

Table 2. Influence of feeding *Lactobacillus acidophilus* cells on serum cholesterol levels in pigs on a high cholesterol diet

Group	Cholesterol (mg/dl)		
	Day 0	Day 5	Day 10
Control	52.23	69.10	74.44
L. acidophilus P 47	55.58	72.01	73.48
L. acidophilus RP 32	52.84	61.48	62.29

Anticancer effects

The anticancer benefits of fermented foods were regarded as folklore, with no scientific backing. But, now there is a strong attestation to the importance of *Lactobacilli* in human nutrition and health, as well as the interrelationship between many dietary factors and cancer[19]. Diets high in animal protein and fat appear to increase the susceptibility to colon cancer, apparently through conversion of procarcinogens to carcinogens by the intestinal microflora. Fats and fried foods also have been implicated in cancers of breast, prostate and pancreas. The consumption of milk has been negatively correlated to the incidence of gastric cancer[20] and has been postulated to play an important role in prevention of human stomach cancer caused by alkylating agents[21]. On the other hand, milk consumption has also been positively correlated with the incidence of colon, prostate and breast cancer[22] and has been attributed to increased consumption of fat, modification of the intestinal flora by milk components, ingestion of milk hormones and presence of an oncogenic virus or other contaminants in milk.

Friend and Sahani[23] induced colon cancer in rats using 1,2-dimethyl hydrazine (DMH). Rats were fed with three types of diets, viz. grains, beef and beef + *L. acidophilus*. They found that the percentage of colon cancer was low with grains diet-fed rats and it was maximum when beef was fed alone. But, cancer incidence was reduced when beef was fed along with *L. acidophilus*.

It is hypothesized that gut microflora, through the production of carcinogens and tumour promoters, are involved in the etiology of colo-rectal cancer. There is some evidence that probiotics can interfere at various stages of the cancer process, such as prevention of DNA damage in the colon by live bacteria[24], suppression of pre-neoplastic changes in the colon[25] and suppression of colon tumours in animals[26].

Studies on the effect of probiotics consumption on cancer appear to be promising[27]. Animal and *in vitro* studies indicate that probiotic bacteria may reduce colon cancer risk by reducing the incidence and number of tumours. One clinical study showed an increased recurrence-free period in subjects with bladder cancer[28]. Results, however, are too preliminary to develop specific recommendations on probiotic consumption for preventing cancer in humans.

The dietary administration of *B. longum* (1×10^{10} live bacterial cells/d) completely suppressed colon tumours induced by the compound 2-amino, 3-methyl, 3-imidazol (4,5-*f*)quinoline, which is a carcinogen found in human diet[29]. McIntosh *et al.*[26] reported that *L. acidophilus* markedly reduced both the number and size of colon tumours induced by another carcinogen, DMH. Recently, studies by Oberreuther–Moschner *et al.*[30] clearly demonstrated that the existence of direct evidence suggesting probiotic consumption may have a beneficial influence on the events related to colon cancer in humans.

Despite the encouraging results from current studies, there are some discrepancies in our knowledge, the prime one being the mechanisms involved in anticancer effects of probiotics. The changes in gut bacterial enzymes that generate carcinogens and tumour promoters such as NH_3 and secondary bile acids, stimulation of immune surveillance, suppression of inflammatory processes, binding of carcinogens in the gut will have various levels of scientific support[31].

Lactose intolerance

Probiotic strains have also proved to solve the problem of lactose intolerance. Lactose intolerance is a physiological state in human beings where they lack the ability to produce an enzyme named lactase or B-galactosidase. This lactase is essential to assimilate the disaccharide in milk and needs to be split into glucose and galactose. Individuals lacking lactase will not be able to digest milk and it often poses a problem in newborn infants. People with lactose intolerance problem express abdominal discomfort, diarrhoea, cramps, flatulence, nausea, vomiting, etc. Another problem associated with lactose intolerance is calcium deficiency. A person suffering from lactose intolerance will be advised to take non-milk diet. The resident bacteria in the colon ferment undigested lactose, producing acid and gas, causing symptoms such as abdominal pain, bloating and diarrhoea. Yogurt contains less lactose than milk and delays gastric emptying, which partly explains why lactose-intolerant individuals tolerate yogurt. However, yogurt tolerance is mainly due to the supply of lactase activity from the lactic acid bacteria present in the yogurt itself. Evidence shows that bacteria must be live and present in sufficient quantity to be of benefit; yogurts containing 10^8 bacteria/ml are required[32].

Milk is the richest source of calcium and Ca requirement of the body is met only through milk. Hence, a person consuming non-milk diet will naturally develop Ca deficiency, leading to osteoporosis. Birge *et al.*[33] confirmed that lactose deficiency leads to calcium malabsorption and thereby to osteoporosis. Calcium malabsorption may be due to deletion of diets with milk to avoid the complications of lactose intolerance. Calcium absorption is better and more in acidic conditions; hence, if lactose is converted to lactic acid, pH of the gut decreases, i.e. it becomes acidic favouring enhanced absorption of calcium. So, if probiotics are fed to lactose intolerance patients, then milk lactose is hydrolysed by probiotic strains and lactose is assimilated and calcium absorption is also favoured.

Garvie *et al.*[3] conducted an experiment on rats to study this situation. Rats were fed with yogurt and yogurt-fed rats were found to have increased concentration of the enzyme which was of bacterial origin. The authors attributed the enhanced enzyme level to the activity of probiotic strains in yogurt. They also concluded that yogurt can be a best alternative to milk.

Allergy

A change in the proper functioning of the immune system can present itself as an allergy. Large-scale studies have indicated an alteration in the composition of the gut microflora, such as decrease in the numbers of lactobacilli, preceding the development of an allergy. Probiotics have been shown to reduce the incidence of childhood eczema by half, compared to placebo, when administered during pregnancy and up to 6 months postnatally[34]. The incidence of asthma or rhinitis was not altered. A follow-up study demonstrated a two-fold increase in transforming growth factor β2, an anti-inflammatory cytokine, in the breast milk of mothers receiving probiotics compared to placebo. Moreover, the reduction in risk of atopic eczema in children whose mothers received probiotics compared to placebo (15 versus 47%) was still evident at 2 years[35].

Probiotics may exert a beneficial effect on allergic reaction by improving mucosal barrier function. In addition, probiotics consumption by young children may beneficially affect immune system development. Probiotics such as *Lactobacillus* GG may be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein[36]. Probiotics consumption may thus be a means for primary prevention of allergy in susceptible individuals. This could play a key role in minimizing allergy at a time when the prevalence of allergic disease in Western societies has increased dramatically over the past 40 years.

Other benefits

There is sufficient evidence to demonstrate that constipation can be alleviated using probiotics. Five of the ten studies showed a beneficial effect on constipation, whereas three out of four studies showed an improvement in transit time. In majority of the studies, probiotics tend to have a more pronounced effect than yogurt per se[37].

Attributes of lactic acid bacteria such as anti-microbial agent production and competition with potential pathogens in the gut provided the impetus for investigating a role for probiotics in diarrhoea. Acute gastroenteritis diarrhoea in infancy is normally due to rotavirus and a number of trials have shown a reduction in the duration[38,39] and incidence of diarrhoea[39,40] in response to probiotics. Thus, both a therapeutic and preventive effect of probiotics has been demonstrated. The precise mechanism is not known, but evidence points to stimulation of the immune system.

Patients are often prescribed broad-spectrum antibiotics to treat an infection, but diarrhoea can be an unwelcome gastrointestinal side effect. Eight clinical trials have shown that the incidence of diarrhoea is lower in response to probiotics versus placebo[41]. Thus, there is convincing evidence for the use of probiotics in the prevention of antibiotic-associated diarrhoea. On the other hand, there is no consistent evidence for a protective effect of probiotics in traveller's diarrhoea, which can be due to a wide range of viruses and bacteria.

Inflammatory bowel diseases such as Chron's disease and ulcerative colitis/pouchitis are chronic and painful. Mixtures of concentrated probiotics have been shown to decrease the number of patients relapsing with pouchitis compared to placebo, over 12- and 18-month periods[42].

Status of probiotics in India

In India, probiotics are often used as animal feed supplements for cattle, poultry and piggery. This requirement is also met by importing probiotics from other countries. It is rarely used for human beings – Sporolac, *Saccharomyces boulardii* and yogurt (*L. bulgaricus* + *L. thermophillus*) are the most common ones. Sporolac is manufactured using Sporolactobacilli. Lactobacilli solution is an example of a probiotic, usually given to paediatric patients in India. The latest and recent addition to the list of probiotics in India is ViBact (which is made up of genetically modified *Bacillus mesentricus*), which acts as an alternate to B-complex capsules. In India, only sporulating lactobacilli are produced and they are sold with some of the antibiotic preparations.

Conclusion

Today probiotics are available in a variety of food products and supplements. In the United States, food products containing probiotics are almost exclusively dairy products – fluid milk and yogurt – due to the historical association of lactic acid bacteria with fermented milk. Probiotics are gaining importance because of the innumerable benefits, e.g. treating lactose intolerance, hypercholesterol problem, cardiac diseases and managing cardiac problems like atherosclerosis and arteriosclerosis. With the current focus on disease prevention and the quest for optimal health at all ages, the probiotics market potential is enormous. Health professionals are in an ideal position to help and guide their clients toward appropriate prophylactic and therapeutic uses of probiotics that deliver the desired beneficial health effects. There are many probiotic products at the market place and most have supporting evidence behind the advertized health claims. New legislation governing the labelling of probiotics, such as indicating the species, strain and number of bacteria present is likely to come into force in the near future. Probiotics should not be considered a pana-

cea for health, but can be incorporated into a balanced and varied diet to maximize good health.

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