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REVIEW ARTICLE

Probiotics and their fermented food products are beneficial for health

S. Parvez[1]*, K.A. Malik[2], S. Ah Kang[3] and H.-Y. Kim[1]

1 Helix Pharms Co. Ltd, Kyung-Hee University, and Department of Biological Sciences of Oriental Medicine, Graduate School of Interdepartmental Studies, Institute of Oriental Medicines, Kyung-Hee University, Dongdaemoon-gu, Seoul, Korea
2 PAEC, Islamabad, Pakistan
3 Department of Molecular Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Gwangjin-gu, Seoul, Korea

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Correspondence
Hong-Yeoul Kim, Institute of oriental Medicine, Kyung-Hee University, Hoegi-dong, Dongdaemoon-gu, Seoul 130-701, Korea.
E-mail: hyk@khu.ac.kr

*Permanent address: NIBGE, P.O. Box 577, Faislabad, Pakistan.

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Abstract

Probiotics are usually defined as microbial food supplements with beneficial effects on the consumers. Most probiotics fall into the group of organisms' known as lactic acid-producing bacteria and are normally consumed in the form of yogurt, fermented milks or other fermented foods. Some of the beneficial effect of lactic acid bacteria consumption include: (i) improving intestinal tract health; (ii) enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients; (iii) reducing symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals; and (iv) reducing risk of certain cancers. The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase. Selection criteria, efficacy, food and supplement sources and safety issues around probiotics are reviewed. Recent scientific investigation has supported the important role of probiotics as a part of a healthy diet for human as well as for animals and may be an avenue to provide a safe, cost effective, and 'natural' approach that adds a barrier against microbial infection. This paper presents a review of probiotics in health maintenance and disease prevention.

Introduction

In the late 19th century, microbiologists identified microflora in the gastrointestinal (GI) tracts of healthy individuals that differed from those found in diseased individuals. These beneficial microflora found in the GI tract were termed probiotics. Probiotics, literally meaning 'for life', are micro-organisms proven to exert health-promoting influences in humans and animals (Marteau *et al.* 1995).

The role(s) of probiotics bacteria in dairy fermentations is to assist in: (i) the preservation of the milk by the generation of lactic acid and possibly antimicrobial compounds; (ii) the production of flavour compounds (e.g. acetaldehyde in yoghurt and cheese) and other metabolites (e.g. extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; (c) to improve the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins; and (iv) the provision of special therapeutic or prophylactic properties as cancer (Reddy *et al.* 1973; Fernandes *et al.* 1987; Gilliland 1990; O'Sullivan *et al.* 1992) and control of serum cholesterol levels (Lin *et al.* 1989). Potential benefits may result from growth and action of the bacteria during the manufacture of cultured foods (Shahani and Ayebo 1980). A therapeutic benefit also includes prophylaxis against some types of intestinal infection (Fernandes *et al.* 1987),

improved digestion of lactose against lactose maldigestion lactose-containing foods (Sawada *et al.* 1990). Lactose malabsorption may compromise their intake of protein and calcium (Saavedra *et al.* 1994; Saikali *et al.* 2004) and these microflora are capable of providing numerous health benefits beyond basic nutritional value (Alm 1982; Hosono 1986; Benchimol and Mack 2004). Figure 1 and Table 1 indicate the summarized form of probiotics and their effect on health.

Metchnikoff (1908) was perhaps the first researcher to propose that fermented dairy products have beneficial properties. During the past two decades there has been renewed interest in the study of the nutritional and therapeutic aspects of these products. While numerous researchers (Taranto *et al.* 1998; Lee *et al.* 1999; Danone 2001) have suggested that lactic cultures and their fermented products provide several nutritional and therapeutic benefits to the consumers (Lilly and Stillwell 1965; Gorbach *et al.* 1987; Mallett *et al.* 1989; De Vuyst and Vandamme 1994; Aso *et al.* 1995; Goldin *et al.* 1996; Campieri and Gionchetti 1999; Lee *et al.* 1999; Bengmark 2000; Caplan and Jilling 2000; Cunningham-Rundles *et al.* 2000; Gionchetti *et al.* 2000a; Gorbach 2000; Guslandi *et al.* 2000; Kyne and Kelly 2001; Reid *et al.* 2001; Reid and Bruce 2001a; MacFarlane and Cummings 2002). The majority of the papers suggest that the potential benefit following the consumption of fermented dairy products containing viable lactic acid bacteria (Deeth and Tamime 1981; Fernandes *et al.* 1987; Gilliland 1990; Fujiwara *et al.* 1997; Gill and Guarner 2004) is primarily attributable to the favourable alteration in GI microecology.

As a result, the development and consumption of functional foods, or foods that promote health beyond providing basic nutrition, are on the rise. Sport bars, soy-based ice cream, cholesterol-reducing margarines and calcium-fortified orange juice are examples of functional food products that have thrived in this era.

Probiotics – live microbial cultures consumed for health benefits beyond providing basic nutritional value. They cooperatively maintain a delicate balance between the GI tract and immune system (Agerholm-Larsen *et al.* 2000) and prebiotics – nondigestible food ingredients that

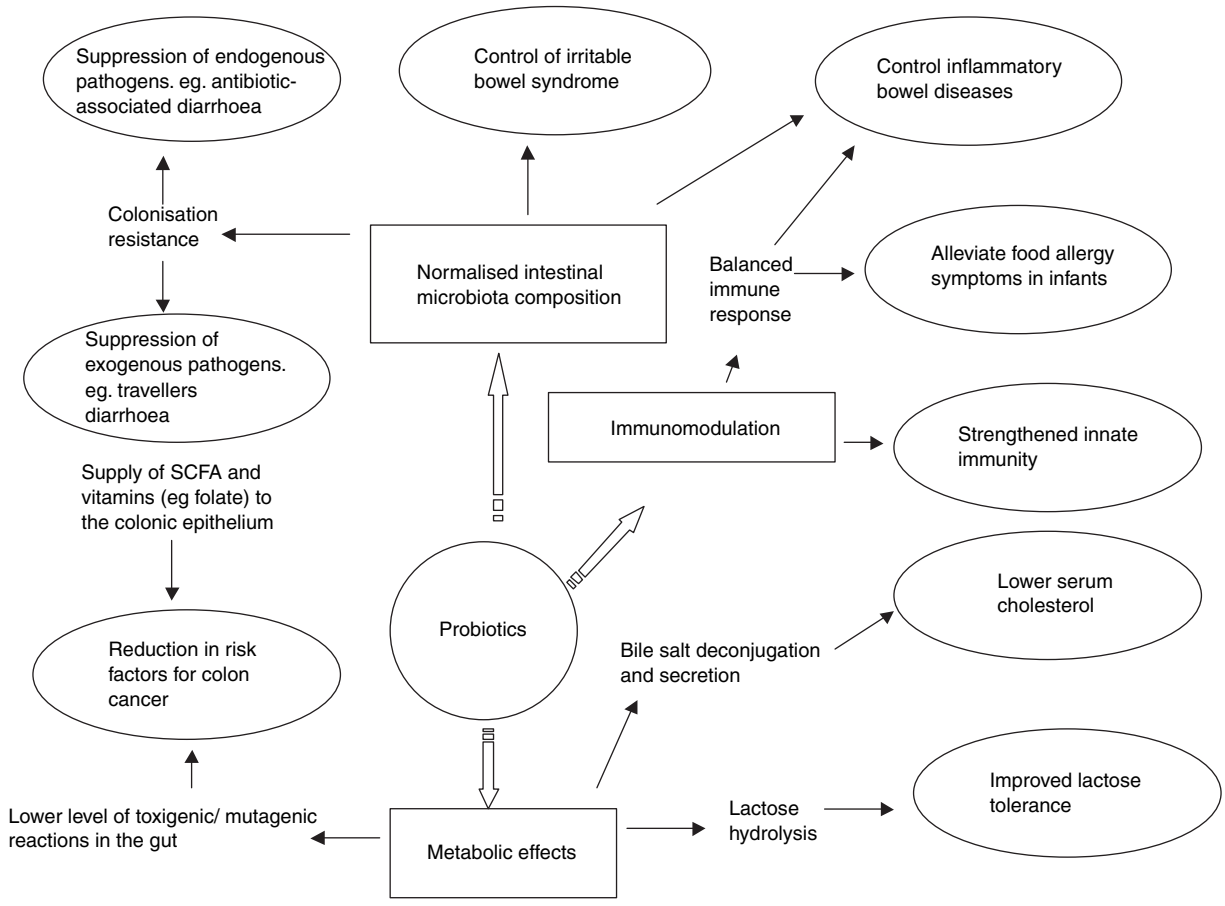

Figure 1 Various health benefits from probiotics consumption.

Table 1 Various special therapeutic or prophylactic properties of specific probiotics

Microflora	Associated actions	Reference
Bifidobacteria species	Reduced incidence of neonatal necrotizing enterocolitis	Caplan and Jilling (2000)
Enterococcus faecium	Decreased duration of acute diarrhoea from gastroenteritis	Marteau *et al.* (2001)
Lactobacillus strains	Administration of multiple organisms, predominantly *Lactobacillus* strains shown to be effective in ameliorating pouchitis	Vanderhoof (2000)
	Lactose digestion improved, decreased diarrhoea and symptoms of intolerance in lactose intolerant individuals, children with diarrhoea, and in individuals with short-bowel syndrome	Marteau *et al.* (2001)
	Microbial interference therapy – the use of nonpathogenic bacteria to eliminate pathogens and as an adjunct to antibiotics	Bengmark (2000)
	Improved mucosal immune function, mucin secretion and prevention of disease	Schultz and Sartor (2000); MacFarlane and Cummings (2002)
Lactobacillus acidophilus	Significant decrease of diarrhoea in patients receiving pelvic irradiation	Marteau *et al.* (2001)
	Decreased polyps, adenomas and colon cancer in experimental animals	Gorbach *et al.* (1987)
	Prevented urogenital infection with subsequent exposure to three ropathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	Sanders and Klaenhammer (2001)
	Lowered serum cholesterol levels	Ouwehand *et al.* (2002)
Lactobacillus plantarum	Reduced incidence of diarrhoea in daycare centres when administered to only half of the children	Vanderhoof (2000)
	Especially effective in reducing inflammation in inflammatory bowel; e.g., enterocolitis in rats, small bowel bacterial overgrowth in children, pouchitis	Schultz and Sartor (2000); Vanderhoof (2000)
	Reduced pain and constipation of irritable bowel syndrome	Vanderhoof (2000)
	Reduced bloating, flatulence, and pain in irritable bowel syndrome in controlled trial.	Nobaek *et al.* (2000)
	Positive effect on immunity in HIV+ children	Walker (2000)
Lactobacillus reuteri	Shortened the duration of acute gastroenteritis	Marteau *et al.* (2001)
	Shortened acute diarrhoea	Shornikova *et al.* (1997a, 1997b)
Lactobacillus rhamnosus	Enhanced cellular immunity in healthy adults in controlled trial	Tomioka *et al.* (1992)
Lactobacillus salivarius	Suppressed and eradicated *Helicobacter pylori* in tissue cultures and animal models by lactic acid secretion	Aiba *et al.* (1998)
Bacteroides species	Chronic colitis, gastritis, arthritis (increased bacterial urease activity in chronic juvenile arthritis)	Vanderhoof (2000)
Saccharomyces boulardii (yeast)	Reduced recurrence of *Clostridium difficile* diarrhoea	Pochapin (2000)
	Effects on *C. difficile* and *Klebsiella oxytoca* resulted in decreased risk and/or shortened duration of antibiotic-associated diarrhoea	Marteau *et al.* (2001)
	Shortened the duration of acute gastroenteritis	Marteau *et al.* (2001)
	Decreased only functional diarrhoea, but not any other symptoms of irritable bowel syndrome	Marteau *et al.* (2001)

encourage the growth and activity of favourable intestinal bacteria are quickly gaining attention as functional foods.

This paper will also review the history and literature of probiotics widely recognized health effects and provide the practicing clinician some basics on interpreting these findings in order to answer clients' questions and make appropriate recommendations.

The probiotics industry is flourishing, and interest in establishing scientific credibility has attained importance for many companies and scientists. Fundamental knowledge of intestinal bacteria and their interactions with each other and with the host are a prerequisite for successful probiotic research and development.

Work of probiotics

To understand how probiotics work, it is important to understand a little about the physiology, microbiology of GI tract and the digestive process. The digestive process begins as soon as food enters the mouth and to stomach, the microbes present in the GI tract have the potential to act in a favourable, a deleterious or a neutral manner.

Microbes in small intestine and in the large intestine complete the digestion process.

Certain intestinal microbes are known to produce vitamins and they are nonpathogenic, their metabolism is non-putrefactive, and their presence is correlated with a healthy intestinal flora. The metabolic end products of their growth are organic acids (lactic and acetic acids) that tend to lower the pH of the intestinal contents, creating conditions less desirable for harmful bacteria. Probiotics may also influence other protective functions of the intestinal mucosa including synthesis and secretion of antibacterial peptides, mucins. The GI tract also serves as a large mucosal surface that bridges the gap between 'inside the body' and 'outside the body'. Along this mucosal interface, microbes and foreign antigens colonizing or passing through the GI tract interact with important components of the immune system. This interaction serves to prime or stimulate the immune system for optimal functioning. Normal microbial inhabitants of the GI tract also reinforce the barrier function of the intestinal lining, decreasing 'translocation' or passage of bacteria or antigens from the intestine into the blood stream. This function has been suggested to decrease infections and possibly allergic reactions to food antigens. Table 1 is the representation of various function activity of probiotics.

History

The concept of probiotics evolved around 1900, when Nobel Prize-winning Elie Metchnikoff hypothesized that the long, healthy lives of Bulgarian peasants were the result of their consumption of fermented milk products and later he was convinced that yogurt contained the organisms necessary to protect the intestine from the damaging effects of other harmful bacteria. The first clinical trials were performed in the 1930s on the effect of probiotics on constipation. In the 1950s, a probiotic product was licensed by the United States Department of Agriculture as a drug for the treatment of scour (*Escherichia coli* infection) among pigs (Orrhage *et al.* 1994). Over the last century, different micro-organisms have been used for their ability to prevent and cure diseases, leading to the coining of the term probiotics (Lidbeck *et al.* 1992; Lee *et al.* 1999).

The discovery by Mann and Spoerig (1974) that people who drank yogurt fermented with wild strains of *Lactobacillus* sp. had very low values for blood serum cholesterol opened up a new area of study. Harrison *et al.* 1975 reported that cells of *Lactobacillus acidophilus* added to infant formula decreased levels of serum cholesterol, and Gilliland *et al.* (1985); Buck and Gilliland (1994), and Gilliland and Walker (1989); Gill and Guarner (2004), showed control of serum cholesterol levels in adult human experiments. In 1994, the World Health Organization deemed probiotics to be the next-most important immune defence system when commonly prescribed antibiotics are rendered useless by antibiotic resistance (Kailasapathy and Chin 2000; Levy 2000). The use of probiotics in antibiotic resistance is termed as a microbial interference therapy.

Composition of probiotic preparations

The most commonly used organisms in probiotic preparations are the lactic acid bacteria (see Table 2), these are found in large numbers in the gut of healthy animals and are in the words of the America FDA, Generally Regarded as Safe.

Organisms other than lactic acid bacteria, which are currently being used in probiotic preparations, include *Bacillus* sp., yeasts (e.g. *Saccharomyces cerevisiae* and *Saccharomyces boulardii*) and filamentous fungi (e.g. *Aspergillus oryzae*) (see Tables 1 and 2). Probiotic products are now available in different formulations with *L. acidophilus*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Enterococcus faecium* and others with or without probiotic and fructooligosaccharides (FOS). Some of the most common probiotic products are *L. acidophilus* with FOS, *L.*

Table 2 The most commonly used species of lactic acid bacteria in probiotic preparations

Lactobacillus sp.	*Bifidobacterium* sp.	*Enterococcus* sp.	*Streptococcus* sp.
L. acidophilus	*B. bifidum*	*Ent. faecalis*	*S. cremoris*
L. casei	*B. adolescentis*	*Ent. faecium*	*S. salivarius*
L. delbrueckii ssp. (*bulgaricus*)	*B. animalis*		*S. diacetylactis*
L. cellobiosus	*B. infantis*		*S. intermedius*
L. curvatus	*B. thermophilum*		
L. fermentum	*B. longum*		
L. lactis			
L. plantarum			
L. reuteri			
L. brevis			

acidophilus and *Bifidus longum* with FOS, and *Bifidus infantis* and *L. acidophilus* with FOS.

These probiotic preparations may be presented in the form of powders, tablets, capsules, pastes or sprays depending on the animal or human receiving the supplement and the condition to be treated.

Selection of probiotics

The selection criteria for a lactic acid bacteria to be used as 'probiotic' include the following ability to: (i) exert a beneficial effect on the host; (ii) withstand into a foodstuff at high cell counts, and remain viable throughout the shelf-life of the product; (iii) withstand transit through the GI tract; (iv) adhere to the intestinal epithelium cell lining and colonize the lumen of the tract; (v) produce antimicrobial substances towards pathogens; and (vi) stabilize the intestinal microflora and be associated with health benefits. Probiotics must have a good shelf-life in food or preparations, containing a large number of viable cells at the time of consumption, and be nonpathogenic and nontoxic in their preparation. The most extensively studied and widely used probiotics are the lactic acid bacteria, particularly the *Lactobacillus* and *Bifidobacterium* spp. mentioned in Tables 1 and 2.

Health benefit and therapeutic effects of probiotics

There are a variety of proposed beneficial health effects of probiotics; only a few have significant research to back up the claims and will be discussed in this paper. Clinical symptoms that have been reportedly treated or have the potential to be treated with probiotics include diarrhoea, gastroenteritis, irritable bowel syndrome, and inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis), cancer, depressed immune function, inadequate lactase digestion, infant allergies, failure-to-thrive, hyperlipidaemia, hepatic diseases, *Helicobacter pylori* infections, and others. The use of probiotics should be further investigated for its possible benefits and its side-effects if any (Bengmark 2000; Benchimol and Mack 2004; Brown and Valiere 2004).

Nutrient synthesis and bioavailability

The action of micro-organisms during the preparation of cultured foods or in the digestive tract has been shown to improve the quantity, availability and digestibility of some dietary nutrients. Fermentation of food with lactic acid bacteria increases folic acid in yogurt, bifidus milk and kefir (Rajalakshmi and Vanaja 1967; Shahani and Chandan 1979; Deeth and Tamime 1981; Alm 1982). Similarly, niacin and riboflavin levels in yogurt are increased with fermentation (Deeth and Tamime 1981; Alm 1982).

Lactic acid bacteria are known to release various enzymes and vitamins into the intestinal lumen. This exert synergistic effects on digestion, alleviating symptoms of intestinal malabsorption, and produced lactic acid, which lowers the pH of the intestinal content and helps to inhibit the development of invasive pathogens such as *Salmonella* spp. or strains of *E. coli* (Mallett *et al.* 1989; Mack *et al.* 1999). Bacterial enzymatic hydrolysis may enhance the bioavailability of protein and fat (Fernandes *et al.* 1987) and increase the production of free amino acids, short chain fatty acids (SCFA), lactic acid, propionic acid and butyric acid are also produced by lactic acid bacteria. When absorbed these SCFAs contribute to the available energy pool of the host (Rombeau *et al.* 1990; Rolfe 2000) and may protect against pathological changes in the colonic mucosa (Leavitt *et al.* 1978; Leopold and Eileler 2000). SCFA concentration helps to maintain an appropriate pH in the colonic lumen, which is critical in the expression of many bacterial enzymes and in foreign compound and carcinogen metabolism in the gut (Mallett *et al.* 1989).

In addition to nutrient synthesis, the action of micro-organisms either during the preparation of cultured foods or in the digestive tract can, to a limited extent, improve the digestibility of some dietary nutrients. Several lines of evidence show that the appropriate strain of lactic acid bacteria, in adequate amounts, can alleviate symptoms of lactose intolerance. *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and other lactobacilli used in fermented milk products deliver enough bacterial lactase to the intestine and stomach where lactose is degraded to prevent symptoms in lactase nonpersistent individuals (Kilara and Shahani 1975; Martini *et al.* 1991). Figure 1 is the representation of various functions and health benefits of probiotics.

Gastric and intestinal tract effect of probiotics

There are a number of studies in humans that suggest that lactic acid bacteria can decrease the incidence, duration and severity of some gastric and intestinal illnesses are discussed below.

Preventative and therapeutic effects against diarrhoea

The well-known uses of probiotics is for diarrhoeal diseases prevention and management of acute viral and bacterial diarrhoea as well as the control of antibiotic-associated diarrhoea are areas of significant potential benefit. A number of specific strains, including *Lactobacillus* GG, *Lactobacillus reuteri*, *Sacc. boulardii*, *Bifidobacteria*

spp., and others, have been shown to have significant benefit for diarrhoea (Hilton *et al.* 1977; Isolauri *et al.* 1991; Duffy *et al.* 1994; Gionchetti *et al.* 2000a, 2000b; Gorbach 2000; Guandalini *et al.* 2000; Levy 2000; Saavedra 2000; Danone 2001; Kyne and Kelly 2001; Marteau *et al.* 2001; Benchimol and Mack 2004), travellers' diarrhoea (Hilton *et al.* 1977) and diarrhoea disease in young children caused by rotaviruses (Saavedra *et al.* 1994; Shornikova *et al.* 1997a, 1997b; Vanderhoof 2000). The probiotic species that show the most promise in treating diarrhoea diseases in children include *Lactobacillus* spp., *L. reuteri*, *Lactobacillus casei*, *Sacc. boulardii*, *Bifidobacterium bifidum* and *Strep. thermophilus* (Hilton *et al.* 1977; Savaiano and Levitt 1987; Isolauri *et al.* 1991; Saavedra *et al.* 1994; Salminen *et al.* 1996; Saxelin 1997; Aiba *et al.* 1998; Gionchetti *et al.* 2000a; Gorbach 2000; Pochapin 2000; Shanahan 2001; Solga 2003; Tomas *et al.* 2004). Lactic acid bacteria are known to release various enzymes into the intestinal lumen that exert synergistic effects on digestion, alleviating symptoms of intestinal malabsorption (see Table 1).

In the paediatric population, probiotics appear to benefit viral diarrhoea, possibly by increasing secretary IgA and decreasing viral shedding, suggesting an immunological mechanism.

Probiotics can prevent or ameliorate diarrhoea through their effects on the immune system. Moreover, probiotics might prevent infection because they compete with pathogenic viruses or bacteria for binding sites on epithelial cells (DeSimone 1986; O'Sullivan *et al.* 1992). Probiotics might also inhibit the growth of pathogenic bacteria by producing bacteriocins such as nisin (Dodd and Gasson 1994; del Miraglia and De Luca 2004).

In vitro studies demonstrate probiotic agents inhibit adherence of dysbiotic organisms to intestinal epithelial cells. This inhibition is hypothesized to be mediated through the ability to increase expression of MUC2 and MUC3 intestinal mucins (Mack *et al.* 1999). Bacterial-to-epithelial cell binding is a multistage process, the first stage of which is characterized by an initial interaction of bacteria with the enterocyte layer. Probiotics increase intestinal mucin production, which prevents the attachment of enteropathogens. The attachment could be prevented by steric hindrance (a slight structural difference in the bacterial ligand interfering with proper attachment to the receptor) or through competitive inhibition for attachment sites on mucins mimicking epithelial cell bacterial attachment sites. Enhancement of innate defence mechanisms in the GI tract, such as mucin production, might be preventive or therapeutic, but this remains to be elucidated (Shahani *et al.* 1987; Tomioka *et al.* 1992; Wagner *et al.* 1997; Schultz and Sartor 2000; Steidler *et al.* 2000; Walker 2000).

Alleviation of lactose intolerance

Lactic acid of the yoghurt alleviates the symptoms of lactose intolerance in lactase-deficient individuals. The beneficial effect appears to be a consequence of the lactic acid bacteria in fermented milk increasing lactase activity in the small intestine (Alm 1982; Gilliland and Kim 1984; Fernandes *et al.* 1987; Marteau *et al.* 1990).

Hepatic disease

Hepatic encephalopathy (HE) is a liver disease and its effects can be life threatening. The exact pathogenesis of HE still remains unknown. The probiotics *Strep. thermophilus*, *Bifidobacteria*, *L. acidophilus*, *Lactobacillus plantarum*, *L. casei*, *L. delbrueckii bulgaricus*, and *E. faecum* containing therapeutic effect have multiple mechanisms of action that could disrupt the pathogenesis of HE and may make them superior to conventional treatment and lower portal pressure with a reduction in the risk of bleeding (Nanji *et al.* 1994; Cunningham-Rundles *et al.* 2000; De Santis *et al.* 2000; Gorbach 2000; Guslandi *et al.* 2000; Shanahan 2001; Solga 2003).

Inflammation/arthritis

Probiotic supplementation has both direct and indirect effects. Probiotics exhibit direct effects locally in the GI tract, including modulation of resident bacterial colonies and vitamin production. There are also indirect effects exerted at sites outside the GI tract, including the joints, lungs, and skin. Indirect effects most likely result from an impact on immunity, via changes in inflammatory mediators such as cytokines. Modulation of inflammatory responses may be related to regulating or modulating the immune system both locally in the GI tract.

It is speculated that inflammation associated with rheumatoid arthritis may be modulated by the use of probiotics (Marteau *et al.* 2001). Thirty patients with chronic juvenile arthritis were randomly allocated to receive *Lactobacillus* GG or bovine colostrum for a 2-week period (Malin *et al.* 1997). Immunological and nonimmunological gut defences were investigated in blood and faeces. It has been observed by different researchers that gut defence mechanisms are disturbed in chronic juvenile arthritis and suggested orally administered *Lactobacillus* GG has potential to reinforce mucosal barrier mechanisms in this disorder. When inflammed, the GI tract becomes permeable and serves as a link between inflammatory diseases of the GI tract and extra-inflammatory disorders such as arthritis. Modulation or downregulation of the immune system and subsequent reduction in GI

permeability can result from consuming probiotics (Yukuchi *et al.* 1992; Vanderhoof 2000).

The potential of probiotics to control allergic inflammation at an early age was assessed in a randomized double-blind placebo-controlled study. The results provide the first clinical demonstration of specific probiotic strains modifying the changes related to allergic inflammation. The data further indicate that probiotics may counteract inflammatory responses beyond the intestinal milieu. The combined effects of these probiotic strains will guide infants through the weaning period, when sensitization to newly encountered antigens is initiated (Mack *et al.* 1999; Vanderhoof 2000).

Allergies/eczema

The prevalence of allergic diseases has increased over the last 35–40 years, particularly in Western societies. Although research is preliminary on how probiotics might modulate allergic reaction, they may exert a beneficial effect by improving mucosal barrier function and microbial stimulation immune system (MacFarlane and Cummings 2002). Probiotic bacteria are important in downregulating inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy (Majamaa and Isolauri 1997; Isolauri *et al.* 2000; McFarland 2000; Murch 2001; Isolauri 2004; Pohjavuori *et al.* 2004). Perinatal administration of *Lactobacillus rhamnosus* GG decreased subsequent occurrence of eczema in at-risk infants by one-half (Isolauri *et al.* 2000). In newborn infants, the initial bacteria to colonize the sterile GI tract may establish a permanent niche and have lasting impact on immune regulation and subsequent development of atopic disorders. It was suggested that probiotics may enhance endogenous barrier mechanisms of the gut and alleviate intestinal inflammation, providing a useful tool for treating food allergy (MacFarlane and Cummings 2002; Kalliomaki and Isolauri 2004; del Miraglia and De Luca 2004).

Probiotics may also be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein. Possibly by degrading these proteins to smaller peptides and amino acids, added to the diet of infants on a hydrolysed whey formula decreased the symptoms of atopic dermatitis (Majamaa and Isolauri 1997). Probiotics have also been found to upregulate anti-inflammatory cytokines, such as interleukin-10, in atopic children (Pessi *et al.* 2000). This is seen both as an immunostimulatory effect in healthy subjects and as a downregulation effect of immunoinflammatory responses in hypersensitive patients.

Similarly, in animal models, it has been demonstrated that probiotics reinforce mucosal degradation of antigens by enhancing breakdown of macromolecules (Pessi *et al.* 1998).

HIV and immune function

Children with HIV infections have episodes of diarrhoea and frequently experience malabsorption associated with possible bacterial overgrowth. Administration of *L. plantarum* 299v can be given safely to immunocompromised hosts, may have a positive effect on immune response, and has the potential to improve growth and development. The immune response may further be enhanced when one or more probiotics are consumed together and work synergistically, as seems to be the case when *Lactobacillus* is administered in conjunction with *Bifidobacteria* (Cunningham-Rundles *et al.* 2000).

The effect of probiotics on the immune response has been comprehensively reviewed (Perdigon and Alvarez 1992; Tomioka *et al.* 1992; Malin *et al.* 1997; McCracken and Gaskins 1999; MacFarlane and Cummings 2002; McNaught *et al.* 2005). The majority of evidence from *in vitro* systems, animal models and humans suggests that probiotics can enhance both specific and nonspecific immune responses. These effects are believed to be mediated through activating macrophages, increasing levels of cytokines, increasing natural killer cell activity and/or increasing levels of immunoglobulins (Perdigon and Alvarez 1992; Ouwehand *et al.* 2002). The immune system is extremely complex, involving both cell-based and antibody-based responses to potential infectious agents. Immunodeficiency can result from certain diseases (e.g. cancer, AIDS and leukaemia) or to a lesser extent from more normal conditions such as old age, pregnancy or stress. Autoimmune diseases (e.g. allergies and rheumatoid arthritis) can also occur due to misdirected immune system activity.

Further confirmation of enhanced immunity and increased resistance to infection has been demonstrated in both animals and humans. In the immunodeficient euthymic mouse model, *Lactobacillus* sp. and *Bifidobacteria* decreased disseminated systemic *Candida albicans* (Wagner *et al.* 1997). In addition, in a placebo-controlled trial, children with cystic fibrosis were found to have reduced severity of pneumonia when *Lactobacillus* GG was administered (Goldin *et al.* 1996; Gorbach *et al.* 1987).

Expanding the use of probiotics in immune-compromised patients appears promising. Clinical testing has focused mostly on immune cell levels and not on actual incidence of disease. However, it is important to remember that there has been very limited testing on immune function effects in humans. Thus, it is difficult to extrapolate results from immune function studies to the expected effects on human health.

Hypertension

Preliminary evidence indicates that probiotic bacteria or their fermented products may also play a role in blood pressure control, with animal and clinical studies documenting antihypertensive effects of probiotic ingestion (Nakamura *et al.* 1995, 1996). Elderly hypertensive patients who consumed fermented milk with a starter containing *Lactobacillus helveticus* and *Sacc. cerevisiae* experienced reductions in systolic and diastolic blood pressure (Hata *et al.* 1996). Decreases in systolic and diastolic blood pressure and heart rate of hypertensive patients were administered powdered probiotic cell extracts (Sawada *et al.* 1990; Nakamura *et al.* 1995). There is a critical need for long-term, well-controlled human studies to evaluate the benefit of probiotic consumption on heart disease risk through their effects on hypertension and blood lipid levels. There is a critical need for long-term, well-controlled human studies to evaluate the benefit of probiotic consumption on heart disease risk through their effects on hypertension and blood lipid levels. Considering the current epidemic of heart disease, regular consumption of probiotics may provide a modest prophylactic effect against heart disease (Nakamura *et al.* 1995).

Cancer

In general, cancer is caused by mutation or activation of abnormal genes that control cell growth and division. (A substance that causes a mistake in genes is known as a mutagen.) Most of these abnormal cells do not result in cancer as normal cells usually out-compete abnormal ones. Also, the immune system recognizes and destroys most abnormal cells. Many processes or exposures can increase the occurrence of abnormal cells. Precautions that minimize these exposures decrease the risk of cancer. Among the many potentially risky exposures are chemical exposures. Cancer-causing chemicals (carcinogens) can be ingested or generated by metabolic activity of microbes that live in the GI system. It has been hypothesized that probiotic cultures might decrease the exposure to chemical carcinogens by: (i) detoxifying ingested carcinogens; (ii) altering the environment of the intestine and thereby decreasing populations or metabolic activities of bacteria that may generate carcinogenic compounds; (iii) producing metabolic products (e.g. butyrate) which improve a cell's ability to die when it should die (a process known as apoptosis or programmed cell death); (iv) producing compounds that inhibit the growth of tumour cells; or (v) stimulating the immune system to better defend against cancer cell proliferation.

Another possible explanation could be the preventive effect of probiotics on carcinogenesis is their effect on other bacteria in the intestine. Probiotics might suppress the growth of bacteria that convert procarcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine. The activity of the enzymes that convert procarcinogens into carcinogens is often used as an indicator of the effect of probiotics on the intestinal microflora (Aso and Akazan 1992) and experimental results suggested that consumption of *L. casei* might delay the recurrence of bladder tumours (Aso and Akazan 1992; Aso *et al.* 1995), but this finding awaits confirmation (McCracken and Gaskins 1999). One hypothesis for the prevention or delay of tumour development by lactobacilli is that they might bind to mutagenic compounds in the intestine (Motta *et al.* 1991; Lidbeck *et al.* 1992; Murch 2001; Isolauri 2004), thereby decreasing the absorption of these mutagens. Note that mutagenicity is mainly estimated as mutagenic potency in the *in vitro* Ames *Salmonella* test; effect of mutagens on cancer risk in humans can differ more than a thousand-fold between humans (Hayatsu and Hayatsu 1993; De Vuyst and Vandamme 1994; Kailasapathy and Chin 2000). Intake of freeze-dried *L. casei* for 3 weeks reduced the urinary excretion of mutagens (Hayatsu and Hayatsu 1993).

Colorectal cancer (CRC) is a major cause of death from cancer in the western world. Approximately 70% of CRC is associated with environmental factors, probably mainly the diet. There is interest in the potential protective role of fermented milks containing probiotic cultures against CRC from human, animal and *in vitro* studies (Rowland 2004; Saikali *et al.* 2004). Cohort studies have failed to detect significant effects, but most case–control studies favour a protective role of fermented milks against colon cancer. Interventional studies have shown a shift of intermediate markers of CRC risk in human subjects from a high- to low-risk pattern after ingestion of fermented milks or probiotics (Saikali *et al.* 2004).

Recent study found that the intervention with probiotic yoghurt, which included the strains *L. acidophilus* 145 and *Bifidobacterium longum* 913, significantly lowered faecal water genotoxicity compared with standard yoghurt (Oberreuther-Moschner *et al.* 2004). However, probiotic intervention also increased oxidative damage; this either reflected pro-oxidative activity or stimulation of endogenous defence systems. Dietary supplementation with a strain of *L. acidophilus* significantly suppressed the total number of colon cancer cells in rats in a dose-dependent manner (De Santis *et al.* 2000). Another study showed that *Lactobacillus* GG reduces the incidence and number of tumours in animals artificially induced with colon cancer (Goldin *et al.* 1996). *Bifidobacterium longum* has also been shown to inhibit the incidence of colon, liver, small intestinal and mammary tumours in rats (Orrhage *et al.* 1994) and few human clinical trials conducted, one

showed that *L. casei* consumption (three times per day for 1 year) increased the recurrence free period among subjects with bladder cancer (Aso *et al.* 1995). Lactic acid bacteria and their fermented food products are thought to confer a variety of important nutritional and therapeutic benefits on consumers, including antimutagenic and anticarcinogeinc activity (Friend and Shahani 1984; Fernandes *et al.* 1987; Fernandes and Shahani 1990; Gilliland 1990; De Vuyst and Vandamme 1994; Dodd and Gasson 1994; Gibson 1995; Hata *et al.* 1996; Danone 2001 and Lee *et al.* 2004). Friend and Shahani (1984) reported that anti-cancer activity occurs when extracts of *L. acidophilus*, *L. casei*, and *L. helveticus* are used in treating sarcomas in mice. Shahani and Ayebo (1980) and Shahani (1983) emphasized that *L. acidophilus* super strain DDS1 produced the strongest antitumour activity. Hosono (1986) reported that milk fermented with *L. delbrueckii* ssp. *bulgaricus* exhibited antimutagenic activities against 4NQO, a typical mutagen, and the water extract of dog faeces, a faecal mutagen, *in vitro* assay.

There is some evidence of primary studies that probiotic bacteria reduce cancer risk possibly by counteracting mutagenic and genotoxic effects.

Control of blood cholesterol and hyperlipidaemia

Cholesterol is essential for many functions in the human body. It acts as a precursor to certain hormones and vitamins and it is a component of cell membranes and nerve cells. However, elevated levels of total blood cholesterol or other blood lipids are considered risk factors for developing coronary heart disease. Although humans synthesize cholesterol to maintain minimum levels for biological functioning, diet also is known to play a role in serum cholesterol levels, although the extent of influence varies significantly from person to person. Lactic acid bacteria have been evaluated for their effect on serum cholesterol levels. Clinical studies on the effect of lowering of cholesterol or low-density lipid (LPL) levels in humans have not been conclusive. There have been some human studies that suggest that blood cholesterol levels can be reduced by consumption of probiotic-containing dairy foods by people with elevated blood cholesterol, but in general the evidence is not overwhelming. Perhaps any effect is small and difficult to measure. In addition, it is likely that some strains may demonstrate this affect while others do not.

Accumulating evidence shows that probiotic bacteria may have a beneficial effect on blood lipid levels. One study in hypercholesterolemic mice showed that administration of low levels of *L. reuteri* for 7 days decreased total cholesterol and triglyceride levels by 38% and 40%, respectively, and increased the high-density lipid : LDL ratio by 20% (Taranto *et al.* 1998). Hyperlipidaemic patients who were administered *Lactobacillus sporogenes* experienced a mean 32% reduction in total cholesterol and 35% reduction in LDL cholesterol over a 3-month period (Mohan 1990). In a well-controlled 8-week clinical trial in overweight subjects, daily consumption of 450 ml of yogurt fermented with *Strep. thermophilus* and *E. faecium* resulted in an 8·4% reduction in LDL and an increase in fibrinogen levels (Agerholm-Larsen *et al.* 2000). Because *in vitro* studies have shown that bacteria can remove cholesterol from culture media (Gilliland *et al.* 1985; Gilliland and Walker 1989; Parvez *et al.* 2005) much attention has been given to the cholesterol-lowering potential of probiotics in humans (Hepner *et al.* 1979; Tamime 2002). It is now thought that cholesterol removal from culture media was a result of precipitation of cholesterol with free bile acids, formed in the media because of the activity of the bacterial enzyme bile salt hydrolase (Klaver and Van der Meer 1993; Parvez *et al.* 2005).

The cholesterol-lowering potential of *L. acidophilus* has been most widely studied. Lin *et al.* (1989) performed two studies: a pilot trial without a placebo and a large placebo-controlled trial. In the pilot trial, 23 subjects received tablets containing 3×10^7 CFU *L. acidophilus* (ATCC 4962) and *L. bulgaricus* (ATCC 33409) daily for 16 weeks, whereas 15 subjects received no tablets. Fasting blood samples were taken before and 7 and 16 weeks after the start of the study. Serum cholesterol in the control group remained stable at 4·9 mmol l^{-1}; serum cholesterol in the experimental group decreased from 5·7 to 5·3 mmol l^{-1} after 7 weeks ($P < 0{\cdot}05$) and to 5·4 mmol l^{-1} after 16 weeks ($P < 0{\cdot}05$ compared with baseline and week 7). A second study with a double-blind, placebo-controlled and crossover design did not show a significant effect of lactobacilli on serum cholesterol (Lin *et al.* 1989; Lee *et al.* 1999). Serum cholesterol concentration reduced after consumption of yogurt enriched with a specific strain of *L. acidophilus* and FOS (Schaafsma *et al.* 1998; Sanders and Klaenhammer 2001).

Conditions of the genitourinary tract

In a recent study of bacterial cultures isolated from women with recurrent episodes of bacterial vaginosis, four different strains of lactobacilli demonstrated inhibitory activity against the bacterial species, possibly by producing an acidic environment (McLean and Rosenstein 2000). In addition, a number of observational studies have correlated vaginal health with the presence of lactobacilli (Hayatsu and Hayatsu 1993; McLean and Rosenstein 2000; Reid and Bruce 2001b; Cadieux *et al.* 2002). The colon might thus be a source of beneficial as well as harmful bacteria for the urinary and genital tracts. Controlled clinical studies are needed to substantiate these preliminary findings. Both oral probiotics and

vaginal suppositories of probiotics have been shown to reduce the incidence of recurrent urinary tract infection (McLean and Rosenstein 2000). One study points to vaginal contamination with faecal flora as the possible rationale for the effectiveness of this therapy (Cadieux *et al.* 2002).

Helicobacter pylori infections

Aiba *et al.* (1998) showed *Lactobacillus salivarius* capable of producing high amounts of lactic acid, which can inhibit the growth of *H. pylori in vitro*. It was found that the higher the level of lactic acid production by *Lactobacillus*, the more potent was the effect on reducing *H. pylori*'s urease activity.

When comparing *L. acidophilus*, *L. casei* and *L. salivarius*, *L. acidophilus* specifically was unable to suppress *H. pylori in vivo*, possibly due to a low level of lactic acid production resulting from poor colonization and growth in the stomach (Bazzoli *et al.* 1992). There is some preliminary evidence that probiotic bacteria may inhibit the gastric colonization and activity of *H. pylori*, which is associated with gastritis, peptic ulcers and gastric cancer. *L. salivarius* was found to inhibit *H. pylori* colonization *in vitro* studies as well as in mice (Aiba *et al.* 1998; McFarland 2000; MacFarlane and Cummings 2002). The use of probiotics in the field of *H. pylori* infection has been proposed for improving eradication rate and tolerability and for compliance of multiple antibiotic regimens used for the infection (Bazzoli *et al.* 1992; Filippo *et al.* 2001). An inhibition of *H. pylori* infection was also shown in humans consuming *Lactobacillus johnsonii* (Michetti *et al.* 1999; Marteau *et al.* 2001).

Inflammatory bowel disease

Studies have shown an improvement in symptoms of IBD, pouchitis and ulcerative colitis with consumption of certain strains of lactobacilli (Gorbach *et al.* 1987; Campieri and Gionchetti 1999; Rembacken *et al.* 1999; Caplan and Jilling 2000; Gionchetti *et al.* 2000a; Nobaek *et al.* 2000; Rolfe 2000; Schultz and Sartor 2000; Shanahan 2001; Femia *et al.* 2002; Ouwehand *et al.* 2002). Lactic acid bacteria may improve intestinal mobility and relieve constipation possibly through a reduction in gut pH (Mallett *et al.* 1989; Naidu *et al.* 1999; Leopold and Eileler 2000; Sanders and Klaenhammer 2001). It has also been reported that probiotic combination therapies may benefit patients with IBD (Campieri and Gionchetti 1999; Gionchetti *et al.* 2000a; Schultz and Sartor 2000; Shanahan 2001). *Saccharomyces boulardii* in patients with Crohn's disease was found to extend remission time and reduce relapse rates. Both *Sacc. boulardii* and *Lactobacillus* GG have been reported to increase secretory IgA levels in the gut (Gorbach *et al.* 1987).

Irritable bowel syndrome

Probiotics exhibit a direct effect in the gut in the treatment of inflammatory and functional bowel disorders. In one of the most common functional bowel disorders, irritable bowel syndrome, *L. plantarum* 299v and DSM 9843 strains were shown in clinical trials to reduce abdominal pain, bloating, flatulence, and constipation (Motta *et al.* 1991; Steidler *et al.* 2000; MacFarlane and Cummings 2002). It was also observed that *Sacc. boulardii* decreased diarrhoea in irritable bowel syndrome, but was not effective in alleviating other symptoms of the syndrome (Marteau *et al.* 2001). Recent study showed a potential role of the intestinal microbiota in the modulation of inflammation in the intestine and joints. Normal gut physiology is moulded by the interaction between the intestinal microbiota and the host's GI tissues, including motility, absorption and secretion, and intestinal permeability (Verdu and Collins 2004).

Efficacy and safety of probiotics

In spite of inherent difficulties establishing good measures of probiotic efficacy (Rolfe 2000), studies on lactose intolerance, diarrhoea and colon cancer show that a daily dose of lactic acid bacteria is needed for any measurable effect (Rembacken *et al.* 1999). Unfortunately, the concentration of probiotics in food products varies tremendously and there are currently no national standards of identity for levels of bacteria required in yogurt or other fermented products. Epidemiological data on the safety of dairy products (Reddy *et al.* 1973; Saavedra *et al.* 1994; Rolfe 2000) and a thorough review of the safety data on probiotics (Ouwehand *et al.* 2002) suggests no evidence of probiotics being involved with human infections. However, there always remains the possibility that probiotic consumption can cause infection and that individuals will respond in different ways to a specific strain. The food industry will need to carefully assess the safety and efficacy of all new species and strains of probiotics before incorporating them into food products.

Future implications of probiotics

In spite of the problems with dosage and viability of probiotic strains, lack of industry standardization and potential safety issues, there is obviously considerable potential for the benefits of probiotics over a wide range of clinical conditions. Ongoing basic research will continue to identify and characterize existing strains of probiotics, identifying strain-specific outcomes, determine optimal doses

needed for certain results and assess their stability through processing and digestion.

Gene technology will certainly play a role in developing new strains, with gene sequencing allowing for an increased understanding of mechanisms and functionality of probiotics. In addition to such basic research, industry-centred research will focus on prolonging the shelf-life and likelihood of survival through the intestinal tract, optimizing adhesion capacity and developing proper production, handling and packaging procedures to ensure that the desired benefits are delivered to the consumer.

Over time, new food products containing probiotics will emerge such as energy bars, cereals, juices, infant formula and cheese, as well as disease-specific medical foods. The establishment of standards of identity for probiotic-containing food products will serve to accelerate the development and availability of these food products.

Conclusion

The oral administration of probiotic therapies may be beneficial in a multitude of disorders both inside and outside the GI tract. The direct effects of probiotics in the GI tract are well documented and include upregulation of immunoglobulins such as IgA, downregulation of inflammatory cytokines, and enhancement of gut barrier function. New research evidence supports indirect, systemic effects of probiotics for a widely divergent set of disorders, including atopic disease, immune compromise and vaginal infections. The health professional is in an ideal position to guide the consumer towards appropriate prophylactic and therapeutic uses of probiotics that deliver the desired beneficial health effects.

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