REVIEW

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Beneficial effects of probiotics and prebiotics on human health

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The primary role of a diet is not only to provide enough nutrients to fulfill metabolic requirements of the body but also to modulate various functions of the body. Probiotics, prebiotics and synbiotics are either beneficial microorganisms or substrates that facilitate the growth of these microorganisms which can be suitably harnessed by the food manufacturers and holds considerable promise for health care industry. Regardless of sufficient health benefits of these, there is a need to carry a multidisciplinary approach on safety evaluation as the conventional toxicological approach has various limitations. This article gives an overview of probiotics and prebiotics, their health effects, mode of action, growth and survival in GIT, quality assurance criteria and safety including future prospects.

1. Introduction

The concept of ingesting a mixture of microorganisms for the improvement of health came to light in 1908, when Elias Metchnikoff (1845–1916), a Nobel Laureate, provided evidence that ingested *Lactobacilli* can displace toxin producing bacteria and explained that they can be used to promote health and prolong life. There is a long history of health claims concerning living microorganisms in food, particularly with lactic acid bacteria. The Persian version of the Old Testament (Genesis 18 : 8) stated, "*Abraham owed his longevity to the consumption of sour milk*". In 76 BC, the Roman historian Plinius recommended the administration of fermented milk products for the treatment of gastroenteritis (Bottazi 1983).

The gut is enriched with more than 500 different species of microbes, most of which are non-pathogenic, serving to protect us against diseases and maintain our well being. Due to its resident bacterial flora, the colon becomes one of the most metabolically active organs in the body besides liver. Many scientists (Bohnhoff et al. 1954; Freter 1955 and 1956) had shown that there is a significant role of the intestinal microflora for resistance to disease. Currently this approach of using microorganisms for better healthcare has become the subject of increasing interest (Mason 2001).

There are many proposed definitions for probiotics depending on their mechanisms of action and their effects on human health. The most commonly used definition is "*Probiotics are the microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance*" (Fuller 1989). Recently a wider definition has been proposed for probiotics: "*A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and thus exerting beneficial health effects in the host*" (Schrezenmeir and De Verse 2001).

Prebiotics differ from probiotics in that they do not contain live microbes, but stimulate their growth in the intestine. The term prebiotic can be defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid 1995).

The term synbiotic is used when a product contains both the probiotics and the prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favours the probiotic compound. Thus, synbiotics are defined as a mixture of probiotics and prebiotics that improve the survival and the implantation of live microbial dietary supplements in the GIT, either by stimulating their growth or by metabolically activating them (Schrezenmeir and De Verse 2001).

The probiotics intended for human use in food and pharmaceuticals must meet the following criteria: preferably of human origin, non pathogenic and safe, genetically stable, tolerant to gastric acidity and bile salt. Further, it should remain viable during technological processing, transit through GIT, should posses good self-life and potential health benefits (Huis in't Veld and Short 1996, Chateris 1998).

2. Health benefits of probiotics and prebiotics

Functional foods comprising probiotic bacteria are receiving increasing attention from the scientific community and science funding agencies. According to the International Life Sciences Institute (ILSI) Europe "*A food can be regarded as functional if it is satisfactorily demonstrated to*

Table 1: Commonly used probiotics, prebiotics and synbiotics

Probiotics	
Lactobacilli	Gram Positive Cocci
Lactobacillus acidophilus	*Lactococcus lactis*
L. delbrueckii (subsp *bulgaricus*)	(subsp *cremoris*)
L. brevis	*Enterococcus faecium*
L. cellobiosus	*Streptococcus salivarius*
L. curvatus	(subsp. *thermophilus*)
L. fermentum	*Streptococous diactylactis*
L. plantarum	*Streptococcus intermedius*
L. casei	
L. rhemnosus	Bifidobacteria
L. reuteri	*Bibidobacterium bifidum*
L. gasseri	*B. adolescentis*
	B. animalis
Yeast	*B. infantis*
Sacchromyces boulardii	*B. longum*
S. cerevisiae	*B. thermophilum*
	B. breve
Prebiotics	Synbiotics
Fructo-oligosacharides (FOS)	*Bifidobacteria* + FOS
Galacto-oligosacharides (GOS)	*Bifidobacteria* + GOS
Inulin	*Lactobacilli* + Lactilol
Lactilol	*Lactobacilli* + Inulin
Lactulose	

(Mason 2001; Madsen 2001; Gionchetti et al. 2000)

Table 2: Applications of probiotics and prebiotics

Diarrhoea	Inflammatory bowel disease (IBD)
Lactose intolerance	Irritable Bowel Syndrome (IBS)
Vaginal infections	Cancer
Urinary tract infections	Immune system
Allergic conditions	Serum cholesterol
Intestinal infections	Osteoporosis

affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well being and/or reduction of risk of disease" (Roberfroid 2002).

Some commonly available probiotics, prebiotics and synbiotics are listed in Table 1 and their possible health benefits in Table 2. The potential nutritional advantages of prebiotics and probiotics consist of preventive and sometimes curative effects against certain diseases are as follows.

2.1. Diarrhoea

Beneficial effects of the human gut flora need definitive confirmation and mechanistic explanations. Historically, there has always been an interest in modulating the composition of gut flora such that a more favourable balance of bacteria resides in the gut (Marteau 2001). Probiotics have been reported to be effective in the prevention and treatment of several types of diarrhoea.

2.1.1. Antibiotics associated diarrhoea

Diarrhoea is a common adverse effect of antibiotic therapy and results in microbial imbalance. There is also a higher risk for other infections and a three-fold increase in mortality (Lima 1990; Zaidi 1991; Mc Farland 1998). *Lactobacillus GG* (in yogurt) was shown to reduce the incidence and duration of diarrhoea in healthy men who received erythromycin for seven days (Siitonen 1990). The non-pathogenic lactic acid producing *Enterococus faecium* strain SF68 reduced the incidence of diarrhoea caused by antibiotics (Wunderlich et al. 1989). Several randomized double blind trials demonstrated that *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG and *Enterococcus faecium* strain SF68 were significantly more efficient than placebo in decreasing the risk of diarrhoea in healthy volunteers as well as in patients receiving antibiotics (Marteau et al. 2001, Szajewska et al. 2001, Armuzzi et al. 2001). A product containing *Lactobacillus acidophilus* and *Lactobacillus bulgaris* (Lactinex, BD) showed benefit in one trial (Gotz et al. 1979) but not in another (Tankanow et al. 1990). In another study, *Bifidobacterium longum* in fermented yogurt reduced stool frequency and abdominal comfort in a crossover study involving 10 volunteers receiving erythromycin (Colombel et al. 1987), and a *B. longum-L.acidophilus* yogurt, reduced diarrhoea in 20 volunteers receiving clindamycin (Orrhage et al. 1994).

2.1.2. Traveller's diarrhoea

The risk of traveller's diarrhoea increases when a person is at equilibrium with a certain microflora and diet and is exposed to unfamiliar microorganisms and environment. A study conducted in Egypt showed that persons who took *S. thermophilus*, *L. bulgaricus*, *L. acidophilus* and *B. bifidum,* experienced a reduction in diarrhoea by about 30% (Black et al. 1989). In another study, the risk of diarrhoea in travellers who took *Lactobacillus GG* was 3.9% when compared with 7.4% in the control group (Hilton et al. 1977). However, *L. acidophilus* and *L. fermentum* had no effect in soldiers who moved to Belize in Central America (Katelaris et al. 1995). Similarly, neither *L. acidophilus* nor *Enterococcus faecium* showed any probiotic effects on Austrian tourists (Kollaritsch et al. 1990). This indicates that the effect of probiotics on traveller's diarrhoea depends on the bacterial strain and the destination of the travellers (De Roos et al. 2000).

2.1.3. Acute infantile diarrhoea

Children in developing countries on an average suffer from seven or more diarrhoeal episodes per year (Guerrant et al. 1990). Numerous probiotic agents have been studied for the management of diarrhoeal disease in pediatric patients (Fedrich 2000; Davison and Butler 2000). In particular, the prevention and management of acute viral diarrhoea, the treatment of recurrent *Clostridium difficile* diarrhoea, as well as control of antibiotic associated diarrhoea seem to be the areas of significant potential benefit. Studies were conducted on the efficacy of an infant probiotic formulation containing *Bifidobacterium bifidum* and *Streptococcus thermophilus* against placebo in a hospitalized infant population over a 17-month period. Diarrhoea developed only in 17% of the population who took probiotics as supplement compared to 31% in the placebo group (Saavedra et al. 1994). The probiotic agents are thus becoming an important part of the armamentarium against gastrointestinal problems in infants and children (Saavedra 2000)

2.2. Lactose intolerance

Lactose intolerance can be due to either a congenitial deficiency of the intestinal mucosal enzyme β-galactosidase or a reduction in lactose activity due to intestinal disorders

like gastroenteritis. Such intolerant individuals suffer from severe intestinal distress characterized by bloating, flatulence and abdominal pain upon consumption of unfermented dairy products. However, there is evidence of improved lactose digestion and tolerance with fermented milk products such as yogurt and *Acidophilus* containing live probiotic bacteria (De Vrese et al. 2000; Sanders 1993; Mustopha et al. 1997)

2.3. Vaginal infections

Many studies on probiotics for vaginal infections have been reported, but most of them lack proper design. Although bacterial vaginosis (BV) and candidal vulvovaginitis are common vaginal infections, few trials have evaluated the efficacy of probiotics for these infections. Perhaps, its resistance to spermicides is a most important attribute for the use of probiotic because these agents can destroy the normal flora (McGroarty et al. 1990 and 1992).

Hydrogen peroxide producing strains are belived to be important in vaginal colonization (Eschenbach et al. 1989) and are widely assumed to offer protection against the overgrowth of pathogens. The obvious reason is the availability of oral antimicrobial agents like metronidazole and fluconazole. Nevertheless, effective probiotics that could prevent or help breaking the cycle of recurrence would be extremely helpful. Most probiotic preparations for vaginal use include one or more *Lactobacillus* species. Favorable effects of intravaginal administration of yogurt to 34 women with bacterial vaginosis have been reported (Neri et al. 1993). In another well-designed study, a hydrogen peroxide producing strain of *L. acidophilus* in suppository form was used to treat women with bacterial vaginosis (Hallen et al. 1992). At the end of the six-day treatment regimen, 16 out of 28 treated women had normal wet mount smears, compared with none of the 29 placebo recipients. The treated women also had reduced counts of bacteroids species but unchanged counts of *Gardnerella vaginalis* and *Mobiluncus* species compared with placebo recipients. Most patients suffered from recurrence of bacterial vaginosis after their next menstruation, indicating the need of prolonged treatment.

In another study (Parent et al. 1996), 32 patients with bacterial vaginosis received one or two vaginal tablets containing a hydrogen peroxide producing strain of *L. acidophilus* and 0.03 mg of estriol per day for six days or placebo. Two weeks after therapy, the cure rate in the treatment group was 77% vs 25% in the placebo group ($P < 0.05$).

2.4. Urinary tract infections

Most urinary tract pathogens in women originate in the intestine (Reid et al. 1999). The close proximity of the urethra to the vagina allows probiotic transfer following vaginal application. However, highly adherent strains of probiotic microorganisms would be of paramount importance for use in urinary tract infections. Reid et al. (1992) have studied *Lactobacillus* containing vaginal suppositories for their efficacy in preventing UTI recurrence after antimicrobial treatment. The recurrence rate in patients receiving probiotic suppositories was 21% compared with 47% in patients receiving placebo. Moreover vaginal suppositories containing *Lactobacillus rhamnosus* did not show any therapeutic benefit over placebo when applied twice a week in cystitis prone women (Baerheim et al. 1995).

More work is needed to determine the efficacy of probiotics against UTIs. Frequent long term vaginal application of a probiotic may not be practical. It would be highly desirable to establish an oral use of probiotics with favourable influence on the recurrence rate of UTIs.

2.5. Allergic conditions

Human beings are exposed to a large number of environmental agents in food. The intestinal mucosa is efficient in assimilating these antigens, though high-level antigen exposure during the first few months may predispose an individual to allergic sensitization. The intestinal microflora is an important constituent of the gut mucosal barrier and in the absence of intestinal microflora antigen transport are increased (Kaur et al. 2002). Probiotic bacteria promote endogenous barrier mechanisms in the patients with atopic dermatitis and food allergy (Majaama and Isolauri 1997). A significant decrease in the extent and intensity of atopic dermatitis was reported in infants fed with hydrolyzed whey containing *Lactobacillus GG* as compared to placebo (only hydrolyzed whey). Similar evidence of efficacy of *Lactobacillus GG* and *Bifidobacterium Bb-12* in treatment of atopic eczema has also been reported (Isolauri et al. 2000). These results suggest that probiotics may act as a useful tool in the treatment of food allergy by alleviating intestinal inflammation.

2.6. Inflammatory bowel disease (IBD)

Inflammatory Bowel diseases (IBD) are disorders of unknown cause characterized by chronic or recurrent intestinal inflammation, including ulcerative colitis, Crohn's disease and pouchitis. Fifteen patients with ulcerative colitis who were intolerant or allergic to 5-amino salicylic acid (5-ASA) were treated with the probiotic preparation VSL#3 (using a combination of *Bifidobacterium longum*, *B. breve*, *B infantis*, *Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueki* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*). Seventy five percent of these patients remained in remission after 12 months of treatment (Venturi et al. 1999). In another study (Gionchetti et al. 2000) a group of 40 chronic pouchitis patients were randomly assigned to receive VSL#3 (6 g/day) or placebo for nine months. At the end of the study, the remission period was 85% in the probiotic compared to 0% in the placebo group. It is not clear if all bacterial species present in VSL#3 were involved in effectiveness. Within four months after therapy, all responding patients experienced relapses.

A study with a non-pathogenic strain of *E. coli*, two capsules twice a day ($\Rightarrow 2.5 \times 10^{10}$ viable bacteria per capsule) was found to be as effective as mesalazine (1.4 to 2.4 gm/day) in maintaining remission in patients with ulcerative colitis (Rembacken et al. 1999). In a pilot double blind controlled study of the efficacy of *Saccharomyces boulardii* on symptoms of Crohn's disease, 20 patients with active, moderate disease were provided with *S. boulardii* or a placebo for 7 weeks in addition to standard treatment (Plein and Hotz 1993). A significant reduction in the frequency of bowel movement and the disease activity was observed in the group receiving the probiotic but not in the group receiving placebo.

2.7. Irritable bowel syndrome (IBS)

Several reports on the effects of various probiotics in patients with irritable bowel syndrome have emerged. The majority of these trials was negative or inconclusive. It

seems however, an interesting track as at least two randomized control trials have shown that some probiotics could influence the gastric transit time in healthy humans (Bougle et al. 1999; Marteau et al. 2002).

2.8. Cancer

Probiotics and/or prebiotics may have antimutagenic effects or inhibit mutagenic activity through modulation of carcinogen generation and suppression of tumors (Reddy 1998; Naidu et al. 1999; Wollowskii et al. 2001). *Lactobacillus* species have been referred to degrade carcinogens such as *N*-nitrosamines (Rowland and Grasso 1975). While probiotics containing *Bifidobacteria* reduced colon cancer induced by 1,2-dimethyl hydrazine in mice when used with fructooligosaccharides (Koo and Rao 1991) and inhibited liver and mammary tumors in rats (Reddy and Rivenson 1993). The cell wall of *Bifidobacterium infantis* induces activation of phagocytes, which destroy the growing tumor cells (Sekine et al. 1994). Several studies on Japanese patients (Aso et al. 1995; Aso and Akazan 1991) have indicated that daily intake of *L. casei* delayed the recurrence of bladder tumors. Clinical studies are currently done in Europe to study the effects of probiotics and prebiotics in subjects with colonic adenomas (Marteau and Boutron-Ruault 2002).

2.9. Immune system

Probiotic bacteria may stimulate antibody production, enhance phagocytosis of pathogens and modify the production of cytokines (Koto et al. 1999; Gill et al. 2000). A significant increase in the levels of IgA and anti poliovirus IgA was found in the faeces of children aged 15–31 months who were fed a formula diet containing viable *Bifidobacterium lactis Bb12* for 20 days (Fukushima et al. 1998). *Lactobacillus GG* was used to manage allergic reactions to cows milk and atopic eczema in 31 infants aged 2–16 months (Majamae and Isolauri 1997). *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, in capsule form caused an appreciable change in inflammatory and immunological responses of elderly people (De Simone et al. 1992). Probiotics may modify the structure of potentially harmful antigens and thereby alter the mode of their immunogenicity (Isolauri et al. 2001).

2.10. Cholesterol

For many years, it has been recognized that elevated serum cholesterol is a risk factor associated with atherosclerosis and coronary heart disease, the latter being a major cause of death. Numerous drugs that lower serum cholesterol have been used to treat hypercholesterolemic individuals. However, the undesirable side effects of these compounds have caused concerns about their therapeutic use. Ingestion of probiotic lactic acid bacteria would possibly be a more natural method to decrease serum cholesterol in humans (Taranto et al. 2004). Yogurt (200 ml daily) containing live cultures of *L. acidophilus* reduced the serum cholesterol level of hypercholesterolemic subjects by 2.9% (Anderson and Gillialand 1998). Serum cholesterol levels were reduced by 4.4% in hypercholesterolemic subjects provided with yogurt (375 ml daily) fermented with *L. acidophilus* and fortified fructooligosaccharides (prebiotic) (Schaafsma et al. 1998). However, prebiotics alone may lower serum cholesterol (Delzemne and Williams 2002). Administration of *Lactobacillus reuteri CRL 1098* for one week to hypercholesterolemic subjects caused a decrease in the total serum cholesterol by 38% compared to the control group (Taranto et al. 1998). Strain AD1, a *Lactobacillus* isolate, has been reported to reduce the higher level of serum cholesterol after oral administration to dogs (Strompfova 2004). Several studies show the cholesterol lowering effect of probiotics and prebiotics. Their therapeutic potential is yet to be established. Since the efficacy of probiotics on lowering blood lipids still remains unproven (Gill and Guarner, 2004).

2.11. Mineral metabolism

Prebiotics (non digestible oligosaccharides like oligofructose or lactulose) may have a significant effect on the absorption of minerals like calcium, phosphorus, magnesium, copper, iron and zinc in animal models (Scholz-Ahrens et al. 2001; Scholz-Ahrens and Schrezenmeir 2002). Studies on rats showed improved or higher absorption of calcium (Lopez et al. 2000), copper (Lopez et al. 2000), magnesium (Ohta et al. 1994), iron (Delzenne et al. 1995; Ohta et al. 1995 and 1998), and zinc (Delzenne et al. 1995) but no change in phosphorus absorption (Ohta et al. 1994).

The effect of inulin on the absorption and the balance of calcium, magnesium, iron and zinc following 28-day dietary treatment periods in human volunteers were studied. There was increased calcium absorption and balance but no effect on the metabolism of the other minerals. This effect could enhance bone mineral density, with a consequent reduction in the risk of osteoporosis (Coudray et al.1997).

3. Mechanisms of action of probiotics

A simple concept that has been proposed is that the ingestion of probiotics improved the intestinal and vaginal mi-

Table 3: Cholesterol-lowering effects of probiotics and prebiotics on humans

Product/ Vehicle	Subject	Dose	Duration	Blood Lipid	Author (Year)
WMY	3 M 1F	4 L	12 days	↓ TC	Mann (1977)
SMY	3 M 2F	2 L	12 days	↓ TC	Mann (1977)
PY	6 M 4F	720 ml	4 weeks	↓ TC	Hepner et al. (1979)
Yoghurt	11 M	2 L	3 weeks	↑ TC, ↑ LDLC	Rossouw et al. (1981)
Yoghurt	5 M 16F	750 gm	1 week	↓ TC	Bazzare et al. (1983)
UPY	58 M	200 ml	6 weeks	↓ TC, ↓ LDLC	Agerbaek et al. (1995)
UPY	47 M 43W	200 ml	24 weeks	↓ LDLC	Richelsen et al. (1996)
Fructan/coffee	8 M 10F	8 gm	2 weeks	↓ TC, ↓ LDLC	Yamashita et al. (1984)
Fructan/breakfast	12 M	9 gm	4 weeks	↓ TAG, ↓ LDLC	Brighenti et al. (1999)

WMY: Whole Milk Yoghurt, SMY: Skimmed Milk Yoghurt, PY: Pasteurized Yoghurt, UPY: Unpasteurized Yoghurt, M: Male, F: Female, ↓: Reduced significantly, ↑: Increased significantly, TC: Total Cholesterol, LDLC: LDL-Cholesterol, TAG: Triacylglycerol

Table 4: Mechanism of action of probiotics

Competitive exclusion of enteric pathogens
Production of pathogen inhibitory substances (eg: Lactic acid, Bacteriocin, etc.,)
Inhibition of actions of microbial toxins
Produce toxic metabolites (eg: hydrogen peroxide)
Restore the normal intestinal flora during antibiotic therapy
Stimulation of immunoglobulin A
Neutralization of dietary carcinogens
Trophic effects on intestinal mucosa

(Kaur et al. 2002; Forestier 2001; Lu and Walker 2001; Blum 1999)

croflora thus restricting the growth of pathogens. The gastro intestinal tract (GIT) colonization resistance is the ability of the normal microbial flora to resist the establishment of pathogens; this may be the primary mechanism underlying the beneficial effects of probiotics (Forestier et al. 2001; Lu and Walker 2001). Production of pathogen-inhibitory substances and stimulation of IgA are some other mechanisms of probiotics (Table 4).

4. Survival and growth of probiotics in GIT

The intestinal microflora within a given individual is remarkably stable, although major differences may exist among different persons (Bartram et al. 1994). Nevertheless, administration of probiotics results in certain changes in the microbial profiles and metabolic activities of faeces. Admittedly such changes are minor, yet, when applied to pathologic situations, they are often sufficient to beneficially alter the course of disease. Many studies have concluded that exogenous probiotics do not permanently grow in GIT and in order to utilize them effectively, their regular uptake is necessary (Kimoto et al. 2004; Bezkorovainy 2001; Lankaputhra and Shah 1995). Studies on diarrhoea in adults and infants have shown a survival rate of 20–40% of the selected probiotic strains, the main obstacles to survival being gastric acidity and the action of bile salts (Bezkorovainy 2001). In order to increase the survival rate, formulation techniques like spray drying (Corcoran et al. 2004) and microencapsulation (Guerin et al. 2003; Chandramouli et al. 2004) are employed. Although it is believed that the maximum probiotic effect can be achieved if the organisms adhere to the intestinal mucosal cells, there is no evidence that the exogenously administered probiotics do adhere to the mucosal cells. Instead they seem to pass into the faeces without having adhered or multiplied. Thus, to obtain a continuous exogenous probiotic effect, the probiotic culture must be ingested continually. These probiotics are often co-administered with prebiotics that facilitate the survival and growth of probiotics in GIT (Kaur et al. 2002; Bezkorovainy 2001).

5. Quality assurance

Probiotic bacteria used in food must retain the characteristics for which they are originally selected. These include characteristics for growth and survival during manufacture, after consumption and during their transit through the stomach and small intestine. Acid and bile stability and intestinal mucosal adhesion properties are among the criteria used for the selection of probiotic microbes. The quality control of probiotic cultures in traditional food has relied solely on tests to ensure that an adequate number of viable bacteria should be present in the products throughout their lives. Viability is an important factor, but it is not the sole criterion for quality assurance. To be effective, probiotic strains must have the functional health characteristics including the ability to survive during transit through the stomach, small intestine and colon. *In vitro* test protocols should be adopted to determine the strain's ability to survive under acidic conditions, survive and grow in the presence of bile and to metabolize selective substrates. Molecular techniques should be used to examine the stability of a strain (Tuomola et al. 2001). Adhesion of probiotic bacteria to intestinal epithelial cells is regarded as a prerequisite for beneficial health effects. Human intestinal epithelial cell lines like Caco-2 or HT-29 cells have been extensively used to select adhesive strains *in vitro* (Blum et al. 1999). Adhesion characterization is an important quality-control method for assessing bacterial effects; adhesion has been related to the shortening of duration of diarrhoea, immunogenic effects, competitive exclusion and other health benefits.

6. Safety of probiotics

The probiotic microorganisms available are generally recognized as safe (GRAS) based upon their long history of use in food fermentation. However, there have been occasional reports of bacteriemias and endocarditis associated with *Lactobacillus* generally in debilitated or immuno-suppressed individuals (Elmer 2001).

There is a theoretical risk of transfer of antimicrobial resistance from the probiotic to other microorganisms, which may come in contact with the probiotics. However, many intrinsically vancomycin resistant strains of *Lactobacillus* have a long history of safe use as probiotics. There is no indication that vaconcomycin resistant *Lactobacilli* could transfer resistance to other bacteria (Saavedra 2001).

In recent years, many species were isolated from various types of infective lesions, suggesting the need for safety studies to be conducted on probiotic products (Ishibashi and Yamazaki 2001). Some intestinal bacteria act on proteins and their digested products to produce ammonia, indole, phenols and amines (Drasar and Hill 1974). The use of probiotics must be carefully considered when they are to be used therapeutically in patients with high risk for opportunistic infections or when the gastrointestinal tract is badly damaged (Salminen et al. 1998).

The possible safety evaluation criteria of probiotic bacterial strains for metabolic activity, infectivity, gene transfer and immune function using *in vitro* and *in vivo* methods have to be established. A critical tailored approach to safety evaluation can ensure that potential benefits of probiotics are accessible to consumers (John O'Brian et al. 1999).

Ishibasi and Yamazaki had accessed the safety of probiotics using studies on metabolic activity (enzymatic activity associated with production of toxic substances), platelet aggregating activity, mucous degradation activity and antibiotic resistance (Ishibashi and Yamazaki 2001)

7. Conclusions and future prospectives

There is sufficient scientific evidence to suggest the beneficial role of probiotics and prebiotics in the maintenance of good health and the prevention of infectious and metabolic diseases. Moreover, sales of functional food exceeded 18.2 billion dollars in 2001, growing over 8% of the total US food market. Frontline strategic management predicts sales in excess of 32.7 billion dollars by 2005 (Leighton 2002).

The most common probiotics are the lactic acid bacteria and bifidobacteria, which are used in yogurts and other dairy products. Many probiotics requires refrigeration to maintain viability. Some commercially marketed formulations of pro, pre and synbiotics are listed in Table 5. The effect of refrigeration on the viability of *L. acidophilus* and *Lactobacillus GG* (ATCC53103) strains in cultured buttermilk and yogurt was conducted, which indicated the efficacy of refrigeration in maintaining the viability of these strains (Nighsuwonger et al. 1996). Selection of specific pre and probiotics for a particularly desired effect is important (Bengmark 2002). Legislation may also be an issue and should favour not only the manufacturers but ensure that potential benefits of probiotics are accessible

Table 5: Some marketed probiotics, prebiotics and synbiotics

Probiotics			
Product	Manufacturer	Country	Ingredients
Bifa 15	Eden Foods	USA	*B. longum*
Lactiflora	Dr. Reddy's	India	*L. acidophilus*
Kyo-Dophilus (Tablets)	Wakunaga Probiotics	USA	*L. acidophilus*
Probio plus	Migros	Switzerland	*L. acidophilus*
Colon Clean	Pharmafood	Holland	*L. acidophilus*
LC1 (Fermented Milk)	Nestle/ Chambourcy	France	*L. acidophilus*
Danone Bio	Danone	UK	*L. casei* GG
Vifit	Stassano	Belguim, UK, Germany	*L. casei* GG
Yakult (Fermented Milk)	Yakult	Japan	*L. casei* Shirota
Provie Fruit Drink	Provive	Belgium	*L. plantarum* 299V
Myconip (Vaginal tablets)	Uni – Sankyo	India	Lactic acid bacillus
Sporolac (Tablet)	Uni – Sankyo	India	Lactic acid bacillus
Culturelle	CAG Functional Foods	USA	*L. rhamnosus* GG
Florastor	Biocodex Inc	USA	*S. boulardi*
Primadophilus, Nature's Way, Probiotica	Mc Neil Consumer health care	USA	*L. reuteri*
Lactinex	BD	USA	*L. acidophilus, L. bulgaricus*
Nancy's Yogurt (Low-fat Yogurt)	Nancy's	USA	*L. acidophilus, B. bifidum*
$^1/_2$% Plus Milk	Robinson Dairy	USA	*L. acidophilus, Bifidobacteria*
Flora Grow	Arise & Shine	USA	*B. infantis, B. longum, B. bifidum*
Kyo-Dophilus (Capsules)	Wakunaga Probiotics	USA	*L. acidophilus, B. bifidum, B. longum*
VSL#3	CSL	Italy	3 strains of *Bifidobacteria*, 4 strains of *Lactobacilli*, 1 strain of *S. salivarius* (subsp. *thermophilus*)
TH1 Probiotics	Jarrow Formulas	USA	*B. longum, S. boulardii L. casei, L. plantarum*
Proflora	Chefaro	Germany	*L. acidophilus, L. bulgaricus, S. thermophilus, Bifidobacteria*
Lactisyn (Powder)	Franco-Indian	India	*L. lactis, L. acidophilus, S. thermophilus, S. lactis*
Blumox – LB (Capsules)	BlueCross	India	Lactic acid bacillus, Amoxcillin
Damoxy – GF (Capsules)	Dabour	India	Lactic acid bacillus, Amoxcillin
Novamox – LB (Capsules)	Cipla	India	Lactic acid bacillus, Amoxcillin
Pedmox (Dry Syrup)	Bactolac	India	Lactic acid bacillus, Amoxcillin
Remdrox – LB (Capsules)	Remedies	India	*Lactobacillus*, Cefadroxil
Novaclox LB (Capsules)	Cipla	India	Lactic acid bacillus, Amoxcillin, Cloxacillin
Hipenox LB (Capsules)	Zydus Cadila	India	Lactic acid bacillus, Amoxcillin, Cloxacillin
Flemiklox – LB (Capsules)	FDC	India	Lactic acid bacillus, Amoxcillin, Cloxacillin
Moxy max (Tablets, Capsules)	Pure health	India	Lactic acid bacillus, Amoxcillin, Cloxacillin
Ampilox – LB (Capsules)	Biochem	India	Lactic acid bacillus, Ampicillin, Cloxacillin
Symbiotik (Capsules, tablets)	Le Sante	India	*L. sporogenes*, Amoxcillin, Cloxacillin
Amplus (Capsules)	Jagsonpal	India	*L. sporogenes*, Ampicillin, Cloxacillin
Penmix plus (Capsules)	Dee Pharma	India	*L. sporogenes*, Ampicillin, Cloxacillin
Bicidal plus (Capsule)	Kee Pharma	India	*L. sporogenes*, Ampicillin, Cloxacillin
Z plus L (Capsule)	Aurbindo	India	*L. sporogenes*, Ampicillin, Cloxacillin
Acidophilus	Wakunaga Probiotics	USA	*L. acidophilus*, lipase, protease, amylase, lactase enzymes
Plus 3 Milk (Low-fat Milk)	Land O' Lakes	USA	*L. acidophilus*, *Bifidobacteria*, Vitamin
Cyfolac (Tablets, capsules)	KAPL, India	India	*L.acidophilus*, Folic Acid, Riboflavine
Nutrolin (Tablets)	Cipla, India	India	Lactic acid bacilli, Thiamine, Riboflavine, Nicotinamide, Pyridoxine
Becelac (capsules)	Dr. Reddy's	India	*L. acidophilus*, Thiamine, Vitamin B-2, Vitamin B-6, Vitamin B-12, Vitamin C, Folic acid, Niacinamide, Calcium panthonate
A-B Yoghurt		France	*L. acidophilus, B. bifidum*
Mil-Mil	Yakult	Japan	*L. acidophilus, B. bifidum, B. breve*
Akulk	Yakult	Japan	*L. acidophilus, B. bifidum, B. breve, L. casei* subsp. *casei*
Meiji Bulgaria Yoghurt	Meiji Milk Products	Japan	*L. delbrueckii* subsp. *bulgaricus, S. salivarius* subsp. *Thermophilus*

Table 5: (continued)

Prebiotics			
Product	Manufacturer	Country	Ingredients
Actiline (Spread)	Vandemoortele	Germeny	Inulin
Nutridrink fibre	Nutrica	Norway	Inulin
Sveltesse	Nestle/ Chambourcy	France	Inulin
Vitalinea (Yogurt and Meal)	Danone	UK	Inulin
Menu Minceur	Yoplait	France	Inulin
Lactolax (Syrup)	Samarth	India	Lactulose
Livoluk (Syrup)	Panacea Biotech	India	Lactulose
Mtlac (Suspension)	CFL	India	Lactulose
Taillefine (yogurt)	Danone	Belgium	Oligofructose
Instaslim (Bars and powder)	Omega	France	Oligofructose
Ligne Bifide dietic range (Biscuits, ready meals)	Vivis	France	Oligofructose
Low-sugar sorbet	Thiriet	France	Oligofructose
Aviva (Biscuits, chocolate drink)	Novartis	Switzerland	Oligofructose
Jour apres Jour (Milk)	Lactel	France	Oligofructose, Vitamins
Synbiotics			
Product	Manufacturer	Country	Ingredients
Actimel (Cholesterol control yogurt)	Danone	Belgium	*L. acidophilus*, Oligofructose
Symbalance (Yogurt)	Tonilait	Switzerland	3 strains of *Lactobacilli*, Inulin
Fysiq (Dairy drink)	Mona	Holland	*L. acidophilus*, Inulin
Probiotic plus Oligofructose (Yogurt)	Baurer	Germany	2 strains of *Lactobacilli*, Oligofructose, Inulin
Bifidus balance + FOS	Jarrow	USA	*B. breve, B. longum, B. bifidum, B. infantis*, FOS
Replenish	Innercleanse 2000	Canada	*L. acidophilus, L. brevis L. plantarum, L. bifidum, L. bulgaricus, L. casei L. rhamnousus,* FOS

(Kaur et al. 2002; Sanders 1998; Van Den Driessche and Veereman-Wauters 2002; Kolida et al. 2002; CIMS 2002)

to consumers. Currently in the United States, these products are being positioned in the medical food category (Aarts 1997).

Further research will be focused on finding new class of bacteria. The most advanced molecular methods for monitoring changes in the gut microbial ecosystem should be employed. There should be a focus on the development of probiotics and prebiotics that are specifically designed for individuals, which show specific patterns of microbiotic composition. Using probiotics in combination with food enzymes, vitamins and antibiotics may gain much importance in the future.

Both basic and applied research is urgently needed to assess the health claims made of prebiotics and probiotics, which should further clarify their beneficial effects.

References

Aarts TD (1997) How long will the "medical food" window of opportunity remain open? J Nutraceut Func Med Foods 1: 45–57.

Agerbaek M, Gerdes LU and Richelson B (1995) Hypercholesterolaemic effect of a new fermented milk product in healthy middle aged men. Eur. J Clin Nutr 49: 346–352.

Anderson JW, Gillialand SE (1998) Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol by hypercholesterolemic humans. J Ann Coll Nutr 18: 43–50.

Armuzzi A, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, Cammarota G, Anti M, De Lorenzo A, Pola P, Gasbarrini G, Gasbarrini A (2001) The effect of oral administration of *Lactobacillus GG* on antibiotic associated gastrointestinal side effects during *Helicobactor pylori* eradication therapy. Alimen Pharmacol Ther 15: 163–169.

Aso Y, Akazan H (1992) BLP study group. Prophylatic effect of *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. Urol Int 49: 125–129.

Aso Y, Akazan H, Kotake T, Tsukamoto T, Imai K (1995) BLP study group. Preventive effect of *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double blind trial. Eur Urol 27: 104–109.

Baerheim A, Larsen E, Digranes A (1994) Vaginal application of *Lactobacilli* in the prophylaxis of recurrent lower urinary track infection in women. Scand J Prim Health Care 12: 234–243.

Bartram HP, Scheppach W, Gerlach S, Ruckdeschel G, Kelber E, Kasper H (1994) Does yogurt enriched with *Bifidobacterium longum* affect colonic microbiology and fecal metabolites in healthy subjects? Am J Clin Nutr 59: 428–432.

Bazzare TL, Wu SL and Yuhas JA (1983) Total and HDL cholesterol concentration following yoghurt and calcium supplementation. Nutr Reports Intl 28: 1225–1232.

Bengmark S (2002) Gut microbial ecology in critical illness: is there a role for pre-, pro-, and synbiotics? Curr Opin Crit Care 8: 145–151.

Bezkorovainy A (2001) Probiotics: determinants of survival and growth in the gut. Am J Clin Nutr 73(suppl) 399S–405S.

Black FT, Andersen PL, Orskov J, Orskov F, Gearslev K. Laulund S (1989) Prophylatic efficacy of *Lactobacilli* on travellers diarrhoea. In: Steffen R (ed) Travel medicine. Conference on International Travel Medicine 1, Zurich, Switzerland. Springer, Berlin, p. 333–335.

Blum S, Reniero R, Schiffrin EJ, Crittenden R, Mattila-Sandholm T, Ouewehand AC, Salminen, von Wright A, Saarela M, Saxelin, Collins, Morelli L (1999) Adhesion studies for probiotics: need for validation and refinement. Trends Food Sci Technol 10; 405–410.

Bohnhoff N, Drake BL, Muller CP (1954) Effect of streptomycin on susceptibility of the intestinal tract to experimental *Salmonella* infection. Proc Soc Exp Biol Med 86: 132–137.

Bottazi V (1983) Food and feed production by microorganisms. Biotechnol 5: 315–363

Bougle D, Roland N, Labeurrier F, Arhan (1999) Effect of Propionibacteria Supplementation on faecal Bifidobacteria and segmental colonic transit time in healthy human subjects. Scand J Gastroenterol 34: 144–148.

Brighenti F, Casiraghi MC, Canzi E, Ferrari A (1999) Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. Eur J Clin Nutr 53: 726–733.

Chandramouli V, Kailasapathy K, Peiris P, Jones M (2004) An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. J Microbiol Methods 56: 27–35.

Chateris W, Kelly P, Morelli L, Collins K (1998) Ingredient selection criteria for probiotic microorganisms in functional diary foods. Int J Dairy Technol 51: 123–136.

CIMS, Media Health Care Pvt Ltd, India Vol: 79, 2002.

Colombel JF, Cortot A, Neut C, Romond C (1987) Yogurt with *Bifidobacterium longum* reduces erythromycin induced gastrointestinal effects. Lancet 2: 43.
Corcoran BM, Ross RP, Fitzgerald GF, Stanton C (2004) Comparative survival of probiotic *Lactobacilli* spray-dried in the presence of prebiotic substances. J Appl Microbiol 96: 1024–1039.
Coudray C, Bellanger J, Castighia-Delaward C, Remesy C, Vermorel M, Rayssignuier Y (1997) Effect of soluble or partly soluble dietary fibers supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr 51: 375–380.
Davison GP, Butler RN (2000) Probiotics in paediatric gastrointestinal disorders. Curr Opin Pediatr 12: 477–481.
De Roos NM, Katan MB (2000) Effect of probiotic bacteria on diarrhoea, lipid metabolism and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr 71: 405–411.
De Simone C, Cirardi A, Grassi A, Lambert Gardini S, Tzantzoglou S, Trinchieri V (1992) Effect of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* on gut mucosa and peripheral blood B lymphocytes. Immunopharmacol. Immunotoxicol 14: 331–340.
De Vrese M, Stegelmann A, Richter B. Fenselau S, Laue C, Chrezermeir J (2001) Probiotics compensation for lactose insufficiency. Am J Clin Nutr 73 (suppl): 421S–429S.
Delzemne NM, Williams CM (2002) Prebiotics and lipid metabolism. Curr Opin Lipidol 13: 61–67.
Delzenne N, Aertssens J, Verplactse H, Reccaro M, Roberfrod M (1995) Effect of fermentable fructo-aligosacharides on mineral, nitrogen and energy digestive balance in the rat. Life Sci 57: 1579–1587.
Drasar BS, Hill MJ (1974) Human intentional flora. Academic press, London, p. 72–102.
Elmer GW (2001) Probiotics: "Living drugs". Am J Health-Syst Pharm 58: 1101–1109.
Eschenbach DA, Davick PR, Williams SJ, Kiebanoff SJ, Young-Smith CM, Holmes KK (1989) Prevalence of Hydrogen peroxide producing *Lactobacillus* species in normal women and women with bacterial vaginosis. J Clin Microbiol 27: 251–256.
Fedrich MJ (2000) A bit of culture for children: Probiotics my improve health and fight diseases. J Am Med Assoc 284: 1365–1366.
Forestier C, De Champs C, Vatony C, Joly B (2001) Probiotic activities of *Lactobacillus casei rhamnosus*: *in vitro* adherence to intestinal cells and antimicrobial properties. Microbiol 152: 167–173.
Freter R (1955) The faetal enteric cholera infection in the guinea pig achieved by inhibition of normal enteric flora. J Infect Dis 97: 57–64.
Freter R (1956) Experimental enteric *Shigella* and *Vibrio* infections in mice and guinea pigs. J Exp Med 104: 411–418.
Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T (1998) Effect of probiotic formula on intestinal immunoglobulin A production in healthy children. Int J Food Microbiol 42: 39–44.
Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66: 365–378.
Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota. Introducing the concept of prebiotics. J Nutr 125: 1401–1412.
Gill HS, Rutherfurd KJ, Prasad J, Gopal PK (2000) Enhancement of Natural and acquired Immunity by *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). Br J Nutr 83: 167–176.
Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Migioli M, Campieri M (2000) Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: A double blind, placebo-controlled trial. Gastroenterol 119: 305–309.
Gotz V, Romankiewicz JA, Moss J, Murray HW (1979) Prophylaxis against ampicillin-associated diarrhoea with a *Lactobacillus* preperation. Am J Hosp Pharm 36: 754–757.
Guerin D, Vuillemard JC, Subirade M (2003) Protection of *Bifidobacteria* encapsulated in polysaccharide-protein gel beads against gastric juice and bile. J Food Prot 66: 2076–2084.
Guerrant RL, Hughes JM, Lima NL, Crane L (1990) Diarrhoea in developed and developing countries: magnitude special settings and etiologies. Rev Infect Dis 12(suppl): S41–S50.
Hallen A, Jarstrand C, Pahlson C (1992) Treatment of bacterial vaginosis with *Lactobacilli*. Sex Transm Dis 19: 146–148.
Hepner G, Fried Rjeor SS, Fusetti L and Morin R (1979) Hypocholesterolaemic effect of yoghurt and milk. Am J Clin Nutr 32: 19–24.
Hilton E, Kolakowski P, Singer C, Smith M (1977) Efficacy of *Lactobacillus* GG as a diarrhoeal prevention in travellers. J Travel Med 4: 41–43.
Huis in't Veld J, Short C (1996) Selection criteria for probiotic microorganisms. The royal society of medicine: International consortium and symposium series-6 p. 27–36.
Ishibashi, Yamazaki S (2001) Probiotics and safety. Am J Clin Nutr 73 (suppl): 465S–470S.
Isolauri E, Arvola T, Sutas Y, Maoilanes E, Salminen S (2000) Probiotics in the Management of Atopic Eczema. Clin Exp Allergy 30: 1605–1610.
Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S (2001) Probiotics : effects on immunity. Am J Clin Nutr 73(suppl): 444S–450S.
John O'Brian, Ross Crittenden, Arthur C.Ouwehand, Seppo Salminen (1999). Safety Evaluation of probiotics. Trend Food Sci Tech 10: 410–424.
Katelaris PH, Salam I, Farthing MJG (1995) *Lactobacilli* to prevent travellers diarrhoea? N Eng J Med 333: 1360–1361.
Kaur IP, Chopra K, Saini A (2002) Probiotics: potential pharmaceutical applications. Eur J Pharm Sci 15: 1–9.
Kimoto H, Nomura M, Kobayashi M, Mizumachi K, Okamoto T (2003) Survival of *Lactococci* during passage through mouse digestive tract. Can J Microbiol 49: 707–711.
Kolida S, Tuohy K, Ggibson GR (2002) Prebiotic effects of inulin and oligofructose. Br J Nutr 87(suppl): S193–S197.
Kollaritsch H, Wiedermann G (1990) Travellers diarrhoea among Austrian tourists: epidemiology. Clinical features and attempts of non-antibiotic drug prophylaxis. In: Pasini W (eds.) Proceedings of the Second International Conference on Tourist Health. Rimini: World Health Organization. p. 74–82.
Koo OM, Rao AV (1991) Long term effect of *Bifidobacteria* and Neosugar on precursor lesions of colonic caner in CF1 mice. Nutr Rev 51: 137–146.
Koto I, Tanaka K, Yokokura T (1999) Lactic acid bacterium potentially induces the production of interleukin-12 and interferon gama by mouse splenocytes. Int J Immunopharmacol 21: 121–131.
Lankaputhra WEV, Shah NP (1995) Survival of *Lactobacillus acidophilus* and *bifidobacterium* ssp. In the presence of acid and bile salts. Dairy. Prod. J. 30: 2–7.
Leighton P (2002) The bright future of functional foods & beverages. Nutraceut World 5: 42–48.
Lima NL, Guerrant RL, Kaiser DL Germanson T, Farr BM (1990) A retrospective cohort study of nosocomical diarrhoea as a risk factor for nosocomical infection. J Infect Dis 161: 948–952.
Lopez HW, Coudray C, Levrat-Verny M, Feillet-Coudray C, Demigne C, Remesy C (2000) Fructo oligosacharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. J Nutr Biochem 11: 500–508.
Lu L, Walker WA (2001) Pathogenic and physiologic interactions of bacteria with the gasterointestional epithelium. Am J Clin Nutr 73(suppl): 1124S–1130S.
Madsen KL (2001) The use of probiotics in gastrointestinal disease. Can J Gastroenterol 15: 817–822.
Majaama M, Isolauri E (1997) Probiotics: A Novel approach in the management of food allergy. J Allergy Clin Immunol 99: 179–185.
Mann GV (1977) A factor in yoghurt which lowers cholesteremia in man. Atherosclerosis 26: 335–340.
Marteau P, Boutron-Ruault MC (2002) Nutritional advantages of probiotcis and prebiotics. Br J Nutr 87 (suppl): S153–S157.
Marteau P, Cuillerier E, Meance S, Gerhardt MF, Myara A, Bouvier M, Bouley C, Tondu F, Bommelaer G, Grimand JC (2002) *Bifidobacterium animalis* strain DN-173010 shortens the colon transit time in healthy women: a double blind randomized controlled study. Alimentary pharmacology and therapeutics. Aliment Pharmacol Ther 16: 587–593.
Marteau P, de Vrese M, Cellier CJ, Schrezenmier J (2001) Protection from gasterointestinal diseases with the use of probiotics. Am J Clin Nutr 73(suppl): 430S–436S.
Mason P (2001) Probiotics and prebiotics. Pharm J 266: 118–121.
Mc Farland LC (1998) Epidemiology, risk factors and treatments for antibiotic associated diarrhoea. Dig Dis 16: 292–307.
McGroarty JA, Sobah F, Bruce AW, Reid G (1990) The spermicidal compound nonoxynose-9 increases adhesion of *Candida* species in human epithelial cells *in vitro*. Infect Immun 58: 2005–2007.
McGroarty JA, Tomeczek L, Pond DG, Red G, Bruce AW (1992) Hydrogen peroxide production by *Lactobacillus* species, correlation with susceptibility to the spermicidal compound nonoxynol-9. J Infect Dis 165: 1142–1144.
Mustopha A, Jiang T, Savaino DA (1997) Improvement of Lactose digestion by humans following ingestion of in fermented acidophilus milk: influence of bile sensitivity, lactose transport and acid tolerance of *Lactobacillus acidophilus*. J Dairy Sci 80: 1537–1545.
Naidu ASS, Bidlack R, Clemens RA (1999) Probiotic spectra of lactic acid bacteria. Crit Rev Food Sci Nutr 38: 13–126.
Neri A, Saboh G, Sarma Z (1993) Bacterial vaginosis in pregnancy treated with yogurt. Acta Obstet Gynecol Scand 72: 17–19.
Nighsuwonger BD, Brashears MM, Gilliland SE (1996) Viability *Lactobacillus acidophilus* and *Lactobacillus casei* in fermented milk products during refrigerated storage. J Dairy Sci 79: 212–219.
Ohta A, Baba S, Takizawa T, Adachi T (1994) Effects of fructo oligosaccharides on the absorption of magnesium in the magnesium deficient rat model. J Nutr Sci Vitaminol. 40: 171–180.
Ohta A, Ohtsuki M, Baba S, Takizawa T, Adachi T, Kumure S (1995) Effect of fructooligosacharides on the absorption of iron, calcium and magnesium in iron-deficient anemic rats. J Nutr Sci Vitaminol 41: 281–291.
Ohta A, Ohtsuki M, Vehara M, Hosono A, Hirayama M, Adchi T, Hara H (1998) Dietary fructo oligosacharides prevent post gastronomy anemia and osteopenria on rats. J Nutr 128: 106–110.

Orrhage K, Brismar B, Nord CE (1994) Effects of supplements of B. *longum* and *L. acidophilus* on the intestinal microbiota during administration of clindamycin. Microb Ecol Health Dis 7: 17–25

Parent D, Bossens M, Bayot D, Kirkpatrick C, Graf F, Wilkinson FE, Kaiser RR (1996) Therapy of bacterial vaginosis using exogeniously applied *Lactobacilli acidophili* and a low dose of estriol: a placebo-controlled multicentric clinical trial. Arzneimittelforsch 46: 68–73.

Plein K, Hotz J (1993) Therapeutic effects of *Saccharomyces boulardii* on mild residual symptoms in a stable phase of Crohn's disease with special respect to chronic diarrhoea – a pilot study. Z Gastroenterol 31: 129–134.

Reddy BS(1998) Prevention of colon cancer by pre-and probiotics: evidence from laboratory studies. Br J Nutr 80(suppl): S219–S223.

Reddy BS, Rivenson A (1993) Inhibitory effect of *Bifidobacterium longum* on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline, a food mutagen. Cancer Res 53: 3914–3918.

Reid G, Smeianov U, Bruce AW (1999) Biotheropeutic agents to prevent cystitis in women. In: Elmer GW, McFarland LV, Surawicz CM (eds.) Biotherapeutic agents and infectious diseases. Totowa. NJ: Humana, p. 245–261.

Reid G. Bruce AW, Taylor M (1992) Influence of three day antimicrobial therapy and *Lactobacillus* vaginal suppositories on recurrence of urinary tract infections. Clin Ther 14: 11–16.

Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT (1999) Nonpathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis; a randomized trial. Lancet 354: 635–639.

Richelsen B, Kristensen K, Pedersen SB (1996) Long-term (6months) effect of a new fermented milk product on the level of plasma lipoproteins – a placebo-controlled and double blind study. Eur J Clin Nutr 50: 811–815.

Roberfroid MB (2002) Functional foods: concepts and application to inulin and oligofructose. Br J Nutr 87(Suppl): S139–S143.

Rossouw JE, Burger EM, Van der Vyver P, Ferreira JJ (1981) the effect of skim milk, yoghurt and full cream milk on human serum lipids. Am J Clin Nutr 34: 351–356.

Rowland IR, Grasso P (1975) Degradation of N-nitrosamines by intestinal bacteria. Appl Microbiol 29: 7–12.

Saavedra JM (2000) Probiotics and infectious diarrhoea. Am J Gastroenterol 95: S16–S18.

Saavedra JM (2001) Clinical application of probiotic agents. Am J Clin Nutr 73(suppl): 1147S–1151S.

Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH (1994). Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and sheeting of rotavirus. Lancet 344: 1046–1049.

Salminen S, von Wright A, Morelli L,Marteau P, Brassard D, de Vos W, Fonden R, Saxelin M, Collins K, Mogensen G, Birkeland S E, Mattila-Sandholm T (1998) Demonstration of safety of probiotics – a review. Int J Food Microbiol 44: 147–171.

Sanders ME (1993) Summary of the conclusions from a consensus panel of experts on health attributed on lactic cultures: Significance to fluid milk products containing cultures. J Dairy Sci 76: 1819–1828.

Sanders ME (1998) Development of consumer probiotics for the US market. Br J Nutr 80(suppl): S213–S218.

Schaafsma G, Meuling WJ, Van Dokkum W. Bouley C (1998) Effects of a milk product fermented by *Lactobacillus acidophilus* and with fructo-oligosacharides added on blood lipids in male volunteers. Eur J Clin Nutr 52: 436–440.

Scholz-Ahrens KE, Schaafsma G, Vanden Heuvel EG, Schrezenmeir J (2001) Effects of prebiotics on mineral metabolism. Am J Clin Nutr 73(suppl): 459S–464S.

Scholz-Ahrens KE, Schrezenmeir J (2002) Inulin, oligofrutose and mineral metabolism – Experimental data and mechanisms. Br J Nutr 87(Suppl): S179–S186.

Schrezenmeir J, de Verse M (2001) Probiotics, prebiotics, and synbiotics – approaching a definition. Am J Clin Nutr 73(Suppl): 361S–364S.

Sekine K, Watenabe-Sekine E, Ohta J, Toida T, Tatsuki T, Kawashima T (1994) Induction and activation of tumoricidal cells *in vitro* and in vivo by the bacterial cell wall of *Bifidobacterium infantis*. Bifidobact Microflora 13: 65–77.

Siitonen S, Vapaatalo H, Salminen S, Gordin A, Saxelin M, Wirkberg R, Kirkkola AL (1990) Effect of *Lactobacillus* GG yogurt in prevention of antibiotic associated diarrhea. Ann Med 22: 57–59.

Strompfova V, Laukova A, Ouwehand AC (2004) *Lactobacilli* and *Enterococci*-potential probiotics for dogs. Folia Microbiol 49: 203–207.

Szajewska H, Kotowska M, Mrukowicz JZ, Armanska M, Mikolajczyk W (2001) Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhoea in infants. J Pediatr 138: 361–365.

Tankanow RM, Ross MB, Ertel IJ, Dickinson DG, Mc Cormick LS, Garfinkel JF (1990) A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhoea. DICP Ann Pharmacother 24: 382–384.

Taranto MP, Medic M, Perdigon G, Ruiz Holgado AP, Valdez GF (1998) Evidence of hypocholesterolemic effect *Lactobacillus reuteri* in hypercholesterolemic mice. J Dairy Sci 81: 2336–2340.

Taranto MP, Perdigon G, Medici M, De Valdez GF (2004) Animal model for *in vivo* evaluation of cholesterol reduction by lactic acid bacteria. Methods Mol Biol 268: 417–422.

Tuomola, Crittenden R, Playne M, Isolouri E, Salminen S (2001) Quality assurance criteria for probiotic bacteria. Am J Clin Nutr 73(suppl): 393S–398S.

Van Den Driessche M, Veereman-Wauters G (2002) Functional foods in pediatrics. Acta Gastro-enterol Bel 65: 45–51.

Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M (1999) Impact on the Composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 13: 1103–1108.

Wollowskii I, Rechkemmer G, Pool-Zobel BL (2001) Protective role of probiotics and prebiotics in colon cancer. Am J Clin Nutr 73(Suppl): 451S–455S.

Wunderlich PF, Braun L, Fumagalli I, D'Apuzzo V, Heim F, Karly M, Lodi R, Politte G, Vonbank, Zeltner L (1989) Double blind report on the efficacy of lactic acid producing *Enterococcus* SF 68 in the preventions of antibiotic associated diarrhoea in the treatment of acute diarrhoea. J Int Med Res 17: 333–338.

Yamashita K, Kawai K, Itakura M (1984) Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. Nutr Res 4: 961–966.

Zaidi N, Ortiz RM, Ponce de Leon S, Calva JJ, Ruiz-Palacios G, Camorlinga M, Cervantes LE, Ojeda F (1991) Hospital acquired diarrhea in adults: a prospective case controlled study in Mexico. Infect Control Hosp Epidemol 12: 349–355.