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Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges

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Abstract

Probiotic bacteria are used to treat disturbed intestinal microflora and increased gut permeability which are characteristic to many intestinal disorders. Examples include children with acute rotavirus diarrhoea, subjects with food allergy, subjects with colonic disorders and patients undergoing pelvic radiotherapy and sometimes changes associated with colon cancer development. In all such disease states altered intestinal microflora, impaired gut barrier and different types of intestinal inflammation are present. Successful probiotic bacteria are able to survive gastric conditions and colonize the intestine, at least temporarily, by adhering to the intestinal epithelium. Such probiotic microorganisms appear to be promising candidates for the treatment of clinical conditions with abnormal gut microflora and altered gut mucosal barrier functions. They are also promising ingredients to future functional foods and clinical foods for specific disease states provided that basic requirements for strains and clinical studies are carefully followed.

Introduction

Probiotics are viable bacteria that influence the health of the host in a beneficial manner. The bulk of evidence on probiotic cultures and foods is based on anecdotal reports and poorly controlled studies making the work inconclusive and recommendations difficult. However, evidence is currently accumulating from well designed, randomized and placebo- controlled double blinded studies indicating that a few well characterized lactic acid bacteria strains have documented probiotic health promoting effects when defined doses are administered (Lee & Salminen, 1995). Increasing numbers of colonization and dose-response studies defining the required doses have been published (Saxelin et al., 1995; Wolf et al., 1995).

The mechanisms behind the specific benefits include a few mechanisms of action which are discussed in relation to the strengthening of the gut mucosal barrier: gut microflora modification, adherence to intestinal mucosa with capacity to prevent pathogen adherence or pathogen activation, modification of dietary proteins by intestinal microflora, modification of bacterial enzyme activity, and influence on gut mucosal permeability (Table 1).

The intact intestinal epithelium with the normal intestinal microflora represent a barrier to the movement of pathogenic bacteria, antigens and other noxious substances from the gut lumen. In healthy subjects this barrier is stable protecting the host and providing normal intestinal function (Figure 1). When either the normal microflora or the epithelial cells are disturbed by triggers such as dietary antigens, pathogens, chemicals or radiation, defects in the barrier mechanisms become evident (Figure 1). Altered permeability further facilitates the invasion of pathogens, foreign antigens and other harmful substances (Isolauri, 1995). Disturbed intestinal microflora may lead to diarrhoea, mucosal inflammation or activation of harmful drugs and carcinogens in intestinal contents (Salminen et al., 1996). For future research and the development of new food and clinical applications of probiotic bacteria a thorough understanding of the mechanisms of this barrier system is essential.

Table 1. Desirable properties of probiotic bacteria

Probiotic Strain Characteristics	Functional and technological properties
Human origin	Species dependend health effects and maintained viability; applicability to fermented foods
Acid and bile stability	Survival into the intestine, maintaining adhesiveness; maintenance of flavour and aroma profiles during processing and storage
Adherence to human intestinal cells	Immune modulation, competitive exclusion of pathogens: maintenance of mild acidity throughout storage time, good adicity profile
Colonization of the human intestinal tract	Multiplication in the intestinal tract at least temporarily, immune modulation; maintenance of this ability throughout processing and storage
Production of antimicrobial substances	Pathogen inactivation in the intestine, normalization of gut flora; good storage stability and shelf-life in functional food products
Antagonism agains cariogenic and pathogenic bacteria	Prevention of dental decay and pathogen exclusion, prevention of pathogen adhesion, normalization of gut flora, ability to keep the properties after freeze-drying, drying and other processing methods
Safety in food and clinical use	Accurate strain identification (genus, species), documented safety
Clinically validated and documented health effects	Dose-response data for minimum effective dosage in different products

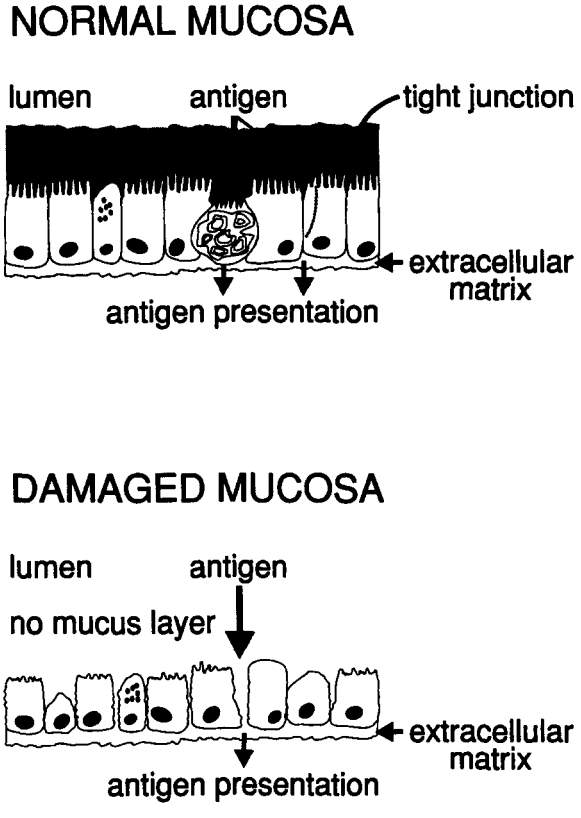

Figure 1. The structure of normal intestinal epithelium and changes caused by outside factors such as diarrhoea, pathogens, radiation and inflammation.

Mucosal and microflora defects and disease

Intestinal barrier

The intestinal mucosa is an important organ of defence providing a barrier against the antigens encountered by the enteric route, and most foreign antigens are excluded by the intestine's mucosal barrier (Sanderson & Walker, 1993). Apart from the barrier function, the intestinal mucosa is efficient in assimilating antigens. For this purpose, there are specialized antigen transport mechanisms in the villous epithelium and particularly in Peyer's patches, essential for evoking specific immune responses (Heyman et al., 1982).

Even in physiological conditions, a quantitatively unimportant but immunologically important fraction of antigens bypasses the defence barrier. They are absorbed across the epithelial layer by transcytosis along two functional pathways (Heyman et al., 1982). The main degradative pathway entails lysosomal processing of the protein to smaller peptide fragments which reduces immunogenicity of the protein and is important in host-defence in diminishing the antigen load. More than 90% of the protein internalized passes in this way. A minor pathway allows the transport of intact proteins which results in antigen- specific immune responses. In health paracellular leakage of macromolecules is not allowed due to intact intercellular tight junctions maintaining the macromolecular barrier. The integrity of the defence barrier is neces-

sary to prevent inappropriate and uncontrolled antigen transport.

Intestinal antigen handling determines subsequent immune responses to the antigen. These include immune exclusion of antigens encountered by the enteric route by interfering with the adherence of antigens, immune elimination of substances that have penetrated the mucosa, and immune regulation of the systemic immune response to antigen-specific systemic hyporesponsiveness (Strobel, 1991). There is evidence that during the absorption process across the intestinal mucosa, antigens are altered into tolerogenic form (Bruce & Ferguson, 1986).

Immature gut defence barrier

The barrier functions are incompletely developed in infancy and early childhood. Intestinal permeability can be transiently increased postnatally, particularly in premature infants (Beach et al., 1982; Axelsson et al., 1989). The binding of antigens to immature gut microvillus membrane is increased compared to the mature mucosa, which has been shown to correlate with the increased uptake of intact macromolecules (Stern et al., 1984). An increased antigen load may evoke aberrant immune responses and lead to sensitization (Holt et al., 1990; Isolauri et al., 1995).

Intestinal inflammation

As a result of local intestinal inflammation, a greater amount of antigens may traverse the mucosal barrier and the routes of transport are altered (Heyman & Desjeaux, 1992). Aberrant antigen transport results in overriding the normal tolerogenic signal into an immunogenic stimulus favouring allergic reactions (Holt, 1994; Fargeas et al., 1995). Foreign antigens such as viruses, bacteria or dietary antigens can induce local inflammation in the intestinal mucosa and, in worst cases, systemic infections and inflammation. Noxious substances in the intestinal contents may also pass the abnormal mucosal barrier. Prior to transport they may be modified by the intestinal microbes and their enzymes (Gorbach, 1990). These functions have in part similar influences on intestinal microflora, intestinal permeability and related factors offering a rational for successful use of lactic acid bacteria for the treatment and prevention of such changes (Figure 2).

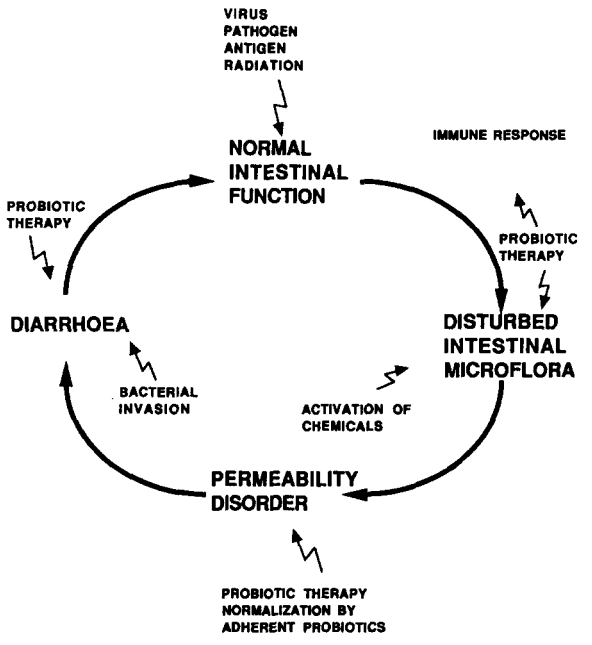

Figure 2. Changes during intestinal disorders and potential targets of treatment and prevention.

Acute gastroenteritis. Rotavirus is the most common cause of acute childhood diarrhoea world-wide (Claeson & Merson, 1990). Rotaviruses invade the highly differentiated absorptive columnar cells of the small intestinal epithelium, where they replicate. Partial disruption of the intestinal mucosa ensues with loss of microvilli and decrease in the villus/crypt ratio. Rotavirus infection has been shown to be associated with increased intestinal permeability (Jalonen et al., 1991). Moreover, the levels of immune complexes containing dietary b-lactoglobulin in sera were significantly higher in patients with rotavirus diarrhoea than in nondiarrhoeal patients. Macromolecular absorption has also been shown to be increased in rotavirus gastoenteritis (Jalonen et al., 1991; Heyman et al., 1987; Uhnoo et al., 1990; Isolauri et al., 1993). The intestinal microflora affects gut permeability, so that in the absence of intestinal microflora, disturbance in intestinal absorption of macromolecules is more severe than in its presence.

Food allergy. Food allergy is defined as an immunologically mediated adverse reaction against dietary antigens. The immaturity of the immune system and the gastrointestinal barrier may explain the peak prevalence of food allergies in infancy (Isolauri & Turjamaa, 1995). In food allergy, intestinal inflammation (Maja-

maa et al., 1995) and disturbances in intestinal permeability (Jalonen, 1991) and antigen transfer (Heyman et al., 1988) occur when an allergen comes into contact with the intestinal mucosa. During dietary elimination of the antigen, the barrier and transfer functions of the mucosa are normal (Majamaa et al., 1995; Jalonen, 1991; Heyman et al., 1988). It has therefore been concluded that impairment of the intestine's function is secondary to an abnormal intestinal immune response to the offending antigens.

Atopic dermatitis. Atopic dermatitis is a common and complex, chronically relapsing skin disorder of infancy and childhood. Hereditary predisposition is an important denominator of atopic dermatitis, and hypersensitivity reactions contribute the expression of this predisposition (Sampson & MacCaskill, 1985). The relationship between environmental allergens and exacerbation of atopic dermatitis is particularly apparent in infancy so that dietary antigens predominate and allergic reactions to foods are common (Isolauri & Turjanmaa, 1995). In a recent study (Majamaa & Isolauri, 1995), macromolecular absorption across the intestinal mucosa was assessed in children (aged 0.5–8 years) with atopic dermatitis. In these patients, the offending foods were identified and eliminated, and the intestinal mucosa was not challenged in vitro nor in vivo. Significantly increased absorption of protein, in intact and degraded form, was found in the atopic dermatitis patients compared to controls. The result may reflect a primarily altered antigen transfer in atopic dermatitis. Aberrant antigen absorption could partly explain why patients with atopic dermatitis frequently show hightened immune responses to common environmental antigens, including dietary antigens.

Crohn's disease. Crohn's disease is a chronic and idiopathic inflammation of the gastrointestinal tract with characteristic patchy transmural lesions containing granulomas. The outbreak of Crohn's disease is thought to require genetic predisposition, immunologic disturbance and the influence of intraluminal triggering agent(s), for example bacteria or viruses. Crohn's disease is associated with impairment of the barrier function. In a recent in vitro study (Isolauri, 1995), a rise in macromolecular absorption in uninvolved parts of the intestine was detected in patients with clinically moderate or severe Crohn's disease. An interplay between the immune effector cells and the intestinal vascular endothelium has been suggested to result in disrupted vasculature, cell-mediated immunity with lymphokine production and a vigorous IgG response and finally dysfunction of the mucosa (Podolsky, 1991; MacDonald, 1993).

Rheumatoid arthritis. Dysfunction of the gut mucosal barrier as a consequence of abnormal intestinal microflora, intestinal inflammation and/ or secondary to diet or medication may be the key connecting the gut and joint in rheumatoid arthritis (Midtvedt, 1987). Evidence for this suggestion is afforded by observations that patients with rheumatoid arthiritis gain relief of clinical symptoms by fasting or specific dietary regimens. In a recent study (Malin et al., 1996), the enzyme activities in faeces were investigated in patients with juvenile chronic arthritis (JCA) and controls. Specifically the activity of urease but not the activities of b-glucosidase and b-glucuronidase in faeces was increased in JCA patients. Urease catalyzes the hydrolysis of urea to yield ammonia. Urease and ammonia may contribute to tissue damage, and elevated urease activity may indicate disturbance in the population of anaerobic bacteria in JCA.

Pelvic radiotherapy. Radiotherapy has a profound effect on the intestinal microflora and mucosa. Radiation alters the intestinal microflora, vascular permeability of the mucosa and intestinal motility (Friberg, 1980; Silva, 1993). The villi are shortened and flattened and there is decreased mitosis in intestinal crypts with necrosis Heavy infiltration of lamina propria with plasma cells is seen with polymorphonuclear leukocytosis (Friberg, 1980; Silva, 1993). The result is a flat surface covered by thin columnar epithelial cells which may also be lost leading to ulcerated surface and appearance of radiation induced fibroblasts. Widening of the tight junctions between cells is common (Silva, 1993). Within three to ten days the intestinal epithelium may be completely denuded and the villous surface is replaced by a layer of exudate in which masses of bacteria are present. Overgrowth of pathogenic microorganisms has been proposed as a factor enhancing the severity of radiation enteritis (Danielsson et al., 1991). Bacteria can penetrate the damaged villi leading to bacteremia in extreme cases (Friberg, 1980; Silva, 1993). The changes leading to radiation enteropathy in man include both damage to intestinal mucosa, changes in the intestinal microflora and impaired immune response (Figure 2).

Clinically, the primary reactions start during the first and second weeks of treatment giving such symptoms as nausea, vomiting and diarrhoea with peritonitis

resulting from the necrotizing effects of radiation. The late secondary reaction, e.g. fibrosis and obstruction of the intestine, may give clinical symptoms years after the treatment. The relationship between early and late reactions is not clear, although some studies have indicated that the severe early reactions precede serious late effects (Silva, 1993; Dahl et al., 1994). *Lactobacillus* supplementation in lethally irradiated mice has been reported to prolong their survival (Dong et al., 1987).

Intestinal inflammation and chemical exposure. Long term inflammatory changes, changes in gut microflora, alterations in drug and carcinogen metabolism, nutrition of intestinal epithelial cells, and genetic factors may alter the ability of intestinal tract to handle drugs and carcinogens. It has been reported in several studies, reviewed by Rogers & Nauss (1985), that nutritional factors such as increasing fat intake, can alter the incidence and number of chemically induced tumours in animals. These changes have been suggested to be related to intestinal microflora, intestinal microbial enzyme activities, intestinal immune response and intestinal mutagen production (Goldin et al., 1996). Subjects with intestinal inflammatory conditions, such as ulcerative colitis and Crohn's disease, may have an increased risk for colon cancer with the risk increasing with the duration and extent of disease (Tytgat et al., 1995). In a study conducted in the United Kingdom, cancer risk was compared in two cohorts of patients with extensive ulcerative colitis and equally extensive colonic Crohn's disease. Both the relative risk and the cumulative incidences of cancer were the same (Gillen et al., 1994).

In the colitic mucosa there may be a spectrum of changes occurring with cycles of inflammation that may contribute to harmful alterations in the mucosa (Tytgat et al., 1995). Inflammatory alterations may be potentiated by the disturbed microflora and its metabolic activities. Intestinal microflora influences the handling of drugs and carcinogens in a healthy subject by assisting the removal of drugs and carcinogens as well as products of enterohepatic circulation (Goldin & Gorbach, 1984; Goldin et al., 1992). These changes may be counteracted by oral administration of suitable lactic acid bacteria either in fermented milks or in other foods.

Probiotic treatment of human intestinal inflammation

Acute gastroenteritis. Lactobacillus GG has been proven effective in the treatment of rotavirus diarrhoea. It repeatedly reduces the duration of diarrhoea to about half in children with rotavirus diarrhoea. It has also been proven effective in watery diarrhoea in several studies in Asia (Raza et al., 1995). One such study has been reported for *Lactobacillus casei* Shirota strain and one prevention study for *Bifidobacterium bifidum* (Sugita & Togawa, 1994; Saavedra et al., 1995).

Different lactic acid bacteria were compared for their effects on the immune response to rotavirus in children with acute rotavirus gastroenteritis (Kaila et al., 1992; Majamaa et al., 1995). Serum antibodies to rotavirus, total number of immunoglobulin-secreting cells (ISC) and specific antibody-secreting cells (sASC) to rotavirus were measured at the acute stage and at convalescence. The treatment with *Lactobacillus* GG was associated with an enhancement of IgA sASC to rotavirus and serum IgA antibody level at convalescence. It was therefore suggested that certain strains of lactic acid bacteria, particularly *Lactobacillus* GG, promote systemic and local immune response to rotavirus, which may be of importance for protective immunity against reinfections (Kaila et al., 1992). Next, a study was made to compare the immunological effects of viable and heat inactivated lactic acid bacteria (Majamaa et al., 1995). *Lactobacillus* GG administered as a viable preparation during acute rotavirus gastroenteritis resulted in a significant rotavirus specific IgA response at convalescence. The heat inactivated *Lactobacillus* GG was clinically as efficient, but the IgA response was not detected. This result suggests that viability of the strain is critical in determining the capacity of lactic acid bacteria to induce immune stimulation. Also, in a study with different preparations of lactic acid bacteria in the treatment of rotavirus diarrhoea it was shown that *Lactobacillus* GG was most effective whilst a preparation containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* or a *Lactobacillus rhamnosus* did not have any effect on the duration of diarrhoea (Majamaa et al., 1995). In a Japanese study, $1{,}5 \times 10^{9-10}$ cfu of Biolactis powder (*Lactobacillus casei* Shirota) was administered to children with rotavirus diarrhoea (n = 17) along with the normal Japanese treatment using lactase and albumin tannate. The controls (n = 15) received lactase and albumin tannate only (Sugita & Togawa 1994). The calculated days until improvement were 3.8 in

the *Lactobacillus* group and 5.3 in the control group ($p < 0.05$). While the result is positive, further studies are required to assess the efficacy using standard biomarkers such as the duration of diarrhoea.

Food allergy. The mechanisms of the immune enhancing effect of *Lactobacillus* GG are not entirely understood, these may relate to the antigen transport in the intestinal mucosa. Therefore, the effect of *Lactobacillus* GG on the gut mucosal barrier was investigated in a suckling rat model (Isolauri et al., 1993). Rat pups were divided into three experimental feeding groups to receive a daily gavage of cow milk, or *Lactobacillus* GG with cow milk, while controls were gavaged with water. At 21 days, the absorption of horseradish peroxidase across patch-free jejunal segments and segments containing Peyer's patches was studied in Ussing chambers. Gut. immune response was indirectly monitored by the ELISPOT method of sASC to b- lactoglobulin. Prolonged cow milk challenge increased macromolecular absorption, whereas *Lactobacillus* GG stabilized the mucosal barrier with a concomitant enhancement of antigen-specific immune defense and proportional transport across Peyer's patches. These results indicate that there exists a link between stabilization of non-specific antigen absorption and enhancement of the antigen-specific immune response. They further suggest that the route of antigen absorption is an important determinant of the subsequent immune response to the antigen (Isolauri et al., 1993).

Atopic dermatitis. The capacity of *Lactobacillus* GG to degrade food antigens, and thereby modify their immunoactivity was investigated (Sütas et al., 1996). For this purpose, the immunoactivity of caseins and *Lactobacillus* GG-degraded caseins was assessed in lymphocyte transformation tests in healthy adults. Casein, as1-casein, b-casein suppressed the lymphocyte proliferation capacity, whereas k-casein induced it. Degradation of casein, as1-casein and b-casein by *Lactobacillus* GG enhanced the suppressive effect of these caseins on the lymphocyte proliferation capacity. Degradation of k-casein by *Lactobacillus* GG reversed its inducive effect to profound suppression. These results indicate that *Lactobacillus* GG can degrade food antigens such as bovine casein, and down-regulate T-cell responses. The results were identical in healthy children and in children with atopic dermatitis (Sütas et al., 1996). Moreover, in cultures of atopic children, casein was found to increase the IL–4 production capacity while *Lactobacillus* GG- degraded casein was found to reduce the IL–4 generation. These results suggest that probiotic bacteria may have the capacity to release tolerogens from allergens.

Crohn's disease. The effect of a ten days' oral bacteriotherapy with *Lactobacillus* GG in Crohn's disease was investigated (Malin et al., 1996b). Irrespective of the activity of Crohn's disease, an increase in IgA sASC to dietary b-lactoglobulin and casein was detected. The result indicates that probiotic bacteria may have the potential to increase gut IgA and thereby promote the gut immunological barrier.

Rheumatoid arthritis. In JCA patients, a ten days' oral bacteriotherapy reduced elevated urease activity in faeces suggesting an effect to counteract imbalanced microflora in JCA (Malin et al., 1996a).

Pelvic radiotherapy. In a randomised study, it was reported that patients receiving pelvic radiotherapy and a fermented milk containing viable *Lactobacillus acidophilus* NCFB 1748 had a significant decrease in diarrhoea (Salminen et al., 1988). In a five-year follow up study there was trend to less late serious intestinal complications (Salminen et al., 1995). It has been shown that *Lactobacillus acidophilus* (NCFB 1748) has ability to colonise human colon mucosa *in vitro* eventhough adhesion to Caco–2 cells is relatively small. In a Swedish study (Henriksson et al., 1995) fermented milk intake decreased the severity of late effects caused by pelvic radiotherapy. This indicates an important role for lactic acid bacteria in the intestinal tract following radiotherapy. Also earlier studies have shown that some *Lactobacillus acidophilus* and *Bifidobacterium* preparations may have potential in controlling diarrhoea and intestinal side effects of radiation (Haller & Kräubig, 1960; Mettler et al., 1973). However, the strains utilized are not described in an up-to-date manner and further studies are required in this area.

Intestinal inflammation, chemical exposure and colon cancer related parameters. There is evidence that lactic acid bacteria influence the mutagenicity of intestinal contents and the levels of faecal microbial enzymes, such as b-glucuronidase, b-glucosidase, nitroreductrase and urease. *Lactobacillus acidophilus* (NCFB 1748) has been shown to significantly decrease faecal mutagenicity and urinary mutagenicity in healthy volunteers consuming fried ground beef. The same strain decreased faecal *E. coli* levels in colon

Table 2. Important studies for the safety assessment of probiotic lactic acid bacteria and other bacteria

Type of property studied	Safety factor to be assessed
Intrinsic properties of lactic acid bacteria	Adhesion factors, antibiotic resistance, existence of plasmids and plasmid transfer potential, harmful enzyme profile
Metabolic products	Concentrations, safety and other effects
Toxicity	Acute and subacute effects of ingestion of large amounts of tested bacteria
Mucosal effects	Adhesion, invasion potential, intestinal mucus degradation, infectivity in immunocompromised animals (e.g. following lethal irradiation)
Dose-response effects	Dose-response studies by oral administration in volunteers
Clinical assessment	Potential for side-effects, careful evaluation in healthy volunteers and disease specific studies
Epidemiological studies	Surveillance of large populations following introduction of new strains and products

cancer patients and reduced the faecal b-glucuronidase levels (Lidbeck et al., 1991). Similar results have been reported for *Lactobacillus* GG, *Lactobacillus acidophilus*, *Lactobacillus casei* Shirota strain and other strains (Ling et al., 1994; Goldin & Gorbach, 1984; Goldin et al., 1992; Morotomi, 1996).

It has also been demonstrated with the *Lactobacillus casei* Shirota strain that there is potential in the area of 'dietary prevention' of cancer. Parenteral administration of the strain has had antitumour and immunostimulating activities on experimentally implanted tumours (Morotomi, 1996). Similar effects have been verivied by oral administration. In a recent study *Lactobacillus* GG was shown to cause a dramatic reduction in DMH induced tumour formation in rats. It was reported that *Lactobacillus* GG inhibited the initiation and early phase promotion on the tumorigenesis process (Goldin et al., 1996). However, it was reported that the organisms was not able to prevent the growth of tumours once they had been established (Goldin et al., 1996). The fact that *Lactobacillus* GG is an organisms of human origin capable of surviving in the gastrointestinal tract and that it inhibits tumour initiation or early promotion in the colon provides a basis for further studies of these factors in humans. In a similar manner, *Lactobacillus casei* Shirota strain has been shown to have inhibitory properties on chemically induced tumours in animals (Morotomi, 1996; Kato et al., 1994). In a clinical study, prophylactic effects of oral administration of *Lactobacillus casei* Shirota on the recurrence of superficial bladder cancer have been reported in two Japanese studies (Aso et al., 1993; Aso et al., 1995). Lactic acid bacteria have also been indicated in colonic fermentation producing butyrate or butyric acid in the colon. This alteration in intestinal metabolism may be due to changes in the intestinal microecology following probiotic intake or the direct metabolism of slowly absorbable components by orally administered colonising lactic acid bacteria. Butyrate has been shown in in vitro studies to slow the growth of cultured colon cancer cells. Young (1996) has proposed that butyrate may be a diet-regulated, natural anti-tumour compound at least partly responsible for the antitumour effect of dietary components and also dietary probiotics. When these studies are related to animal data on several *Lactobacillus* strains and colon cancer related parameters (Table 3), it is important to assess the potential of probiotic lactobacilli for cancer chemoprevention.

Important properties for probiotic bacteria

Adhesion and colonization

Adhesion of lactic acid bacteria to intestinal cells is the first step of colonisation or temporary colonisation. Adhesion can be non-specific, based on physicochemical factors, or specific involving adhesin molecules on the surfaces of adherent bacteria and receptor molecules on epithelial cells. Variability of adhesion properties tested using human intestinal cells has become a standard procedure for selecting new probiotic strains. The adhesion properties of a strain are considered often species specific, but often there appears to be adhesion on non-host species. In general, common dairy strains are not among the most adhesive ones, but probiotic strains appear to have strong adhesion. Based on adhesion, some lactic acid bacteria of human origin and some of dairy origin show moderate to good adhesion properties in human cell lines. *Lactobacillus acidophilus LB*, *Lactobacillus* GG (ATCC 53103),

Table 3. Successful probiotic bacteria and their reported effects

Strain	Reported effects in clinical studies	Selected References
Lactobacillus acidophilus LA1	Immune enhancer, adjuvant, adherent to human intestinal cells, balances intestinal microflora	Link-Am.ster et al., 1994; Bernet et al., 1994; Bernet et al., 1993; Schiffrin et al., 1996
Lactobacillus acidophilus NCFB 1748	Lowering faecal enzyme activity, decreased faecal mutagenicity, prevention of radiotherapy related diarrhoea, treatment of constipation	Lidbeck et al., 1991; Salminen et al., 1988; Sarem-Damerdji et al., 1995; Salminen et al., 1995
Lactobacillus GG (ATCC 53013)	Prevention of antibiotic associated diarrhoea, treatment and prevention of rotavirus diarrhoea, treatment of relapsing *Clostridium difficile* diarrhoea, prevention of acute diarrhoea, Crohn's disease, antagonistic against cariogenic bacteria, vaccine adjuvant	Kaila et al., 1992; Siitonen et al., 1991; Isolauri et al., 1992; Salminen et al., 1993; Majamaa et al., 1995; Raza et al., 1995; Kaila et al., 1995; Meurman et al., 1994
Lactobacillus casei Shirota	Prevention of intestinal disturbances, treatment of rotavirus diarrhoea, balancing intestinal bacteria, lowering faecal enzyme activities, positive effects in the treatment of superficial bladder cancer, immune enhancer in early colon cancer, immune enhancement	Asa et al., 1995; Tanaka & Ohwaki, 1994; Salminen et al., 1993; Sugita & Togawa, 1994; Sawamura et al., 1994; Kato et al., 1994; Okawa et al., 1989
Streptococcus thermophilus; Lactobacillus bulgaricus	No effect on rotavirus diarrhoea, no immune enhancing effect during rotavirus diarrhoea, no effect on faecal enzymes	Majamaa et al., 1995; Goldin et al., 1992
Bifidobacterium bifidum	Treatment of rotavirus diarrhoea, balancing intestinal microflora, treatment of viral diarrhoea	Saavedra et al., 1994; Marteau et al., 1990
Lactobacillus gasseri (ADH)	Fecal enzyme reduction, survival in the intestinal tract	Pedrosa et al., 1995
Lactobacillus reuteri	Colonizing the intestinal tract, Mainly animal studies so far, possibly an emerging human probiotic	Casas et al., 1996; Molin et al., 1993

Lactobacillus acidophilus NCFB 1748, *Lactobacillus reuteri, Lactobacillus rhamnosus* (LA750) and *Lactobacillus acidophilus* BG2FO4 have been shown to be adherent in Caco–2 cells or in other systems (Elo et al., 1991; Chauviere et al., 1992). Temporary colonisation has been reported to *Lactobacillus* GG (ATCC 53103), *Lactobacillus reuteri, Lactobacillus gasseri* ADH and *Lactobacillus acidophilus* LA1 (Goldin et al., 1992; Saxelin et al., 1991; Saxelin et al., 1995).

In clinical studies, adherence and colonisation are thought to be important for health effects. It has been shown in a number of studies that adherent lactic acid bacteria are good candidates for the treatment of acute diarrhoea, radiation gastroenteritis and intestinal inflammation (Salminen et al., 1988; Isolauri et al., 1991). Apart from that, adherent lactic acid bacteria are also thought to be responsible for changes in intestinal microflora, intestinal bacterial enzyme activities and stabilising of intestinal permeability. Thus, adherence studies and colonisation studies form a basis for future probiotic research and adherent strains are the key candidates for succesfull probiotic therapy.

Stability. The most important properties for future probiotics include the acid and bile tolerance, adherence to human intestinal mucosa, temporary colonization of the human gastrointestinal tract, production of antimicrobial substances and inhibition of pathogen growth (Lee & Salminen, 1995). It is also important that the strains used are of human origin since many of the properties may be species dependent (Table 1).

Safety. The safety of lactic acid bacteria used in clinical and functional food is of great importance. In general, lactic acid bacteria have a good record of safety, and no major problems have occurred. Cases of infection have been reported with several strains, most commonly with the ones that are naturally most abundant in the human intestinal mucosa (Gasser, 1994; Aguirre & Collins, 1993). Studies on safety have been document-

ed on dairy strains (Saxelin et al., 1996a; Saxelin et al., 1996b) and a review of current knowledge on safety of probiotic bacteria has been reported by Donohue and Salminen (1996). It is most important for future probiotic lactic acid bacteria that their safety has been assured and that they confirm to all regulations. A proposed scheme for safety assessment is presented in Table 2. (Donohue & Salminen, 1996). Most stringent studies have to be completed for genetically modified strains intended to human consumption.

Successful probiotic strains

Probiotic bacteria with these properties and documented clinical effects include *Lactobacillus acidophilus* (NCFB 1478), *Lactobacillus casei* Shirota strain, *Lactobacillus* GG (ATCC 53103) and *Lactobacillus acidophilus* LA1 (Lee & Salminen, 1995). A large number of published studies exists on each preparation documenting their health effects. All of these are currently further tested for different intestinal problems and they offer alternatives for dietary treatment of intestinal disorders (Table 3). In future, it is likely that we shall see more specific clinical targets for probiotic therapy and then the above mentioned strains are likely to play an important role in new products. New strains emerge and are likely to be included in our diet.

Food and clinical applications of probiotics

Probiotic bacteria (e.g., *Bifidobacterium bifidum* and *Lactobacillus* GG, *Lactobacillus casei* Shirota) have beneficial effects on the clinical course of rotavirus diarrhoea, including prevention and treatment, and the enhanced immune response thereafter (Isolauri et al., 1991; Kaila et al., 1992; Saavedra et al., 1994). In a similar manner, *Lactobacillus acidophilus* preparations and *Lactobacillus casei* (powder prepared from heat killed *Lactobacillus casei* Shirota) preparations have been beneficial in the prevention of radiation enterophathy (Salminen et al., 1988; Salminen et al., 1995; Okawa et al., 1993). *Lactobacillus acidophilus* and *Lactobacillus* GG have enhancing effects on the immune system in connection with oral vaccines and *Lactobacillus reuteri* on the duration of acute diarrhoea in children (Casas et al., 1995). Am.ong the possible mechanisms responsible for the favourable clinical response is promotion of the immunologic and nonimmunologic defence barrier in the gut.

Table 4. Requirements for good clinical studies of demonstration of probiotic properties for functional and clinical use

Each strain documented and tested independently, on its own merit
Extrapolation of data from closely related strains not acceptable
Well-defined probiotic strains, well-defined study preparations
Double-blind, placebo controlled human studies
Randomized human studies
Results confirmed by several independent research groups
Publication in peer-reviewed journals

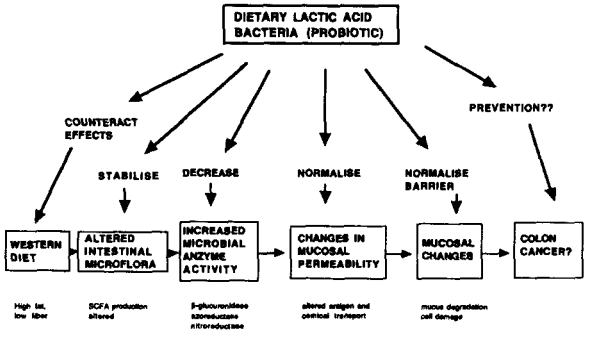

Figure 3. Possible mechanisms of lactic acid bacteria influencing colon cancer related parameters.

Oral introduction of *Lactobacillus acidophilus* LA1 and *Lactobacillus GG* have been associated with alleviation of intestinal inflammation and *Lactobacillus GG* with normalization of increased intestinal permeability (Isolauri et al., 1993) and gut microflora (Isolauri et al., 1994). Such studies have not been reported for the other successful probiotic strains. Another explanation for the gut stabilizing effect of *Lactobacillus GG* could be improvement of the intestine's immunologic barrier, particularly intestinal IgA responses (Isolauri et al., 1993).

The above mentioned health effects and known mechanisms offer a basis for both applications of these strains in functional food products and, in specific disease states, also in clinical foods and medical foods. Due to differences between even closely related strains all strains shouls be tested on their own merit with good clinical study protocols as outlined in Table 4.

Types of clinical food applications

Management in food allergy includes exclusion of responsible food from the diet. In attempts to eliminate cow milk allergens from the diet, the most common approach is predigestion of bovine casein or whey to create formulae that provide nitrogen as mixtures of peptides and amino acids (Isolauri et al., 1995). The data taken together suggest that probiotic bacteria down-regulate hypersensitivity reactions and promote endogenous barrier mechanisms. The use of probiotic bacteria supplemented formula can further reduce antigenicity of substitute formulae and consequently have an important role in the development of specific therapy for patients who have food allergy.

Management of acute diarrhoea requires infant formulae with efficient lactic acid bacteria. Another alternative is to supply oral rehydration solutions with similar bacteria. In clinical nutrition, elemental diets are used for the treatment of intestinal inflammation, radiation enteritis and inflammatory bowel disease. Probiotic lactic acid bacteria do have a role in alleviating these disturbances and thus, the introduction of such strains into clinical foods and special dietary foods is likely. A new area is opened with the possibility of influencing food allergy and a variety of products could be developed for this area using the specific strains.

Another promising area for future research is the prevention and adjuvant treatment of cancer of the colon or bladder. Human studies using *Lactobacillus casei* Shirota strain appear promising in both areas. Other strains, such as *Lactobacillus* GG and *Lactobacillus acidophilus* NFCB 1748 have been beneficial in animal studies with colon carcinogens and in human studies using faecal bacterial enzymes as biomarkers.

Conclusion

The applications described in this paper indicate that there are some common background characteristics for many intestinal disturbances which facilitate effective use of probiotic bacteria. It is clear that probiotic bacteria have potential in the treatment of clinical conditions with altered gut mucosal barrier functions. Probiotic bacteria offer new dietary alternatives for the stabilization of the intestinal microflora. They can be used for immunotherapy to counteract local immunological dysfunctions and to stabilize the natural gut mucosal barrier mechanisms. It is important that the probiotic properties of each strain are demonstrated in carefully planned and controlled human studies.

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