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Gut bacteria and health foods—the European perspective

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Abstract

Probiotics, prebiotics, and synbiotics aimed at improving intestinal health currently represent the largest segment of the functional foods market in Europe, Japan and Australia. Evidence continues to emerge demonstrating that these ingredients have the potential to improve human health in specific intestinal disorders. The European Commission, through its 5th Framework Programme, is presently focusing on a substantial effort in the science of the intestinal microbiota, its interaction with its host and methods to manipulate its composition and activity for the improvement of human health and well being. Eight multicentre and multidisciplinary research projects now cover a range of topics required for the development of efficacious probiotic foods, from understanding probiotic mechanisms at a molecular level; developing technologies to ensure delivery of stable products; and demonstrating safety and efficacy of specific probiotics in defined treatment targets. This concerted research effort promises to provide us with an enhanced understanding of the human intestinal microbiota's role in health and disease, and new approaches and products to tackle a variety of intestinal problems.

Keywords: Probiotics; GI-tract; Indigenous microbiota; Consumer; European research; Health-effects; Chronic diseases; Diarrhoea; IBS; IBD; Arthritis; Allergy

1. Introduction

Foods are no longer considered by consumers only in terms of taste and immediate nutritional needs but also in terms of their ability to provide specific benefits above and beyond their basic nutritional value. Functional foods have become an important and rapidly expanding segment of the food market as processed food manufactures seek to improve market share by promoting the health benefits provided by functional ingredients in their products. Nutritional science has been expanding the knowledge of how foods influence consumers in relation to specific health parameters. Functional foods targeted towards improving the balance and activity of the intestinal milieu currently provide the largest segment of functional food market in Europe, Japan and Australia (Hilliam and Young, 2000; Heasman and Mellentin, 2001).

Ever since yoghurt was proposed as a health promoting food by Metchnikoff in (1907), the oldest and still most widely used way to increase the numbers of advantageous bacteria in the intestinal

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Table 1
Properties of probiotics to be assessed during the development of new strains and new probiotic functional foods

Property	Target and method
Strain specificity	Source or origin to be assessed.
Resistance to pH	Model systems for gastric and bile effects.
Adhesion and colonisation	Several model systems to be used for adhesion (e.g. cell cultures, mucus, intestinal segments). Colonisation in human studies.
Competitive exclusion	Adhesion and competitive exclusion of pathogens in in vitro and in vivo model systems.
Immune regulation	In vitro and human studies.
Safety	Pre-market clearance and post-market surveillance.
Technological properties	Various systems for stability and activity throughout the processes.
Sensory assessment	Sensory testing of model and final products.
Consumer acceptance	Consumer studies on product formulations.
Efficacy assessment	Human clinical intervention studies with final product formulations; at least two independent studies to show efficacy in target populations and safety in all consumer groups.

tract has been the direct consumption of live bacteria. Such bacteria are called probiotics (Fuller, 1989; Salminen et al., 1998a), and have to date been predominantly selected from the genera *Lactobacillus* and *Bifidobacterium*, both of which form part of the normal human intestinal microbiota or the mucosal microbiota. In the probiotic approach, the ingested bacteria are selected to survive gastrointestinal transit

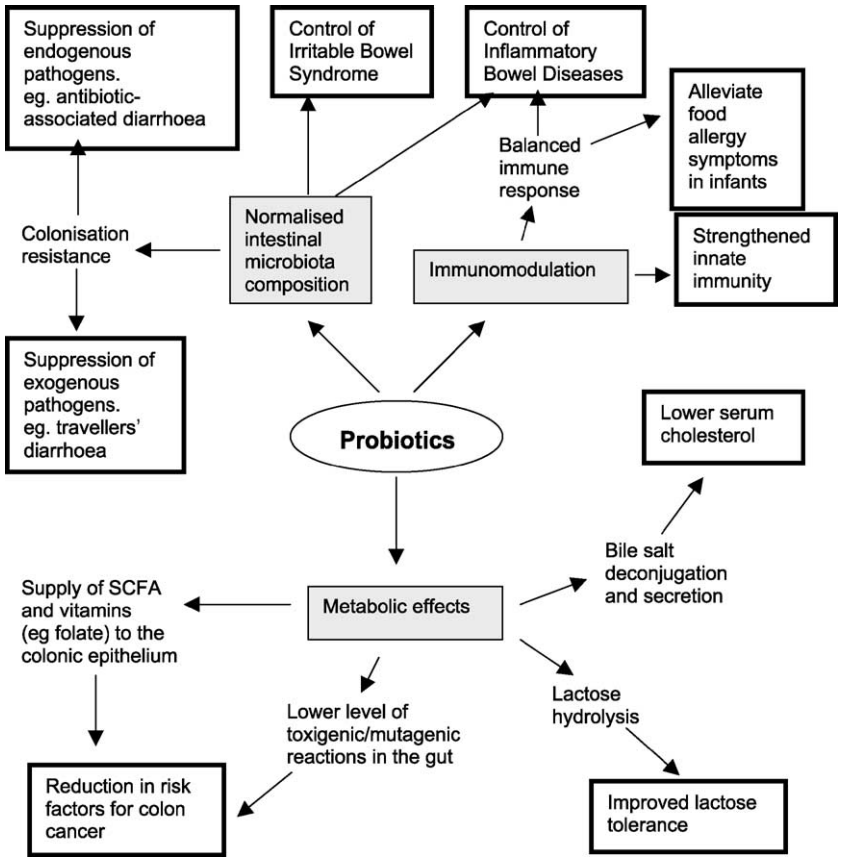

Fig. 1. Proposed health benefits stemming from probiotic consumption.

and arrive viable and able to contribute positively to the activity of the intestinal microbiota, and thus, the health of the host (Table 1). As evidence accumulates for their beneficial effects on human health, these bacteria are increasingly being included as functional ingredients, particularly in dairy products such as yoghurts and other fermented milks (Dunne et al., 1999; Mattila-Sandholm and Saarela, 2000) (Fig. 1).

Prebiotics represent a second strategy to improve the balance of intestinal bacteria. Rather than introducing exogenous strains into an individual's intestinal tract, prebiotics aim to selectively stimulate the proliferation and/or activity of advantageous groups of bacteria already present in the intestinal microbiota. They are non-digestible food ingredients that reach the colon intact, where they can be fermented preferentially by beneficial groups of bacteria. To date, prebiotics have primarily been oligosaccharides and other indigestible carbohydrates that increase the population of strains of *Bifidobacterium* in humans and animals. There is a need to understand prebiotic mechanisms to further develop and characterise these components for functional foods (Salminen et al., 1998a). There is an obvious potential for synergy between prebiotics and probiotics. Hence, foods containing both prebiotic and probiotic ingredients have been termed synbiotics (Gibson and Roberfroid, 1995).

A concerted effort to improve our understanding of relationships between the intestinal microbiota and human health and disease has now been launched in Europe through the European Commission's 5th Framework Programme. This paper reviews the current state of the art in foods for gut health and describes the major European Commission projects involved in the development of functional foods targeting modifications to intestinal microbiota composition and activity for enhanced human health and well being.

2. Gastrointestinal tract microbiota

2.1. Microbiota in different parts of the GI-tract

The normal microbiota of the human gastrointestinal (GI) tract is still a relatively unexplored organ of host defence (Berg, 1996; Gaskins, 1997). The established normal microbiota provides the most important contact with the environment for the host and a barrier against harmful components of the diet as well as against pathogenic bacteria (Benno and Mitsuoka, 1986; Grönlund et al., 2000; Kirjavainen et al., 2001).

Although bacteria are distributed throughout the intestines, the major concentration of microbes and metabolic activity can be found in the large intestine. From culture-based data, it has been demonstrated that the mouth harbours a complex microbiota consisting of facultative and strict anaerobes including streptococci, bacteroides, lactobacilli and yeasts and this microbiota is strongly influenced by dietary and environmental factors (Benno and Mitsuoka, 1986). The upper bowel (stomach, duodenum, and jejunum) has a sparse microbiota with up to 10^5 colony-forming units (CFU)/ml of contents. From the ileum on, bacterial concentrations gradually increase reaching 10^{10} to 10^{11} CFU/g in the colon. It has been further estimated that at least 500 different microbial species exist in the human intestinal microbiota, although on a quantitative basis 10–20 genera probably predominate. Members of the microbiota that have been commonly cultured from faecal samples include isolates of *Bacteroides*, *Clostridium*, *Eubacterium*, *Ruminococcus*, *Fusobacterium*, *Bifidobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Enterococcus*, *Peptococcus*, *Enterobacter* and *Veillonella* (Finegold et al., 1974; Moore and Holdeman, 1974; Benno and Mitsuoka, 1986; Berg, 1996). Using fluorescent in situ hybridisation (FISH), Franks et al. (1998) showed that the numerically dominant bacteria in the faeces belong to *Bacteroides* and the *Clostridium coccoides–Eubacterium rectale* group (representing about 50% of the bacterial community). Similar results were obtained by Sghir et al. (2000): Using oligonucleotide probes they found that *Bacteroides–C. coccoides–E. rectale* comprised about 70% of the faecal bacterial microbiota. In addition, also *Fusobacterium prausnitzii* and related species have been shown to belong to the dominant bacterial microbiota in faeces (Suau et al., 1999). Marteau et al. (2001b) compared the bacterial groups in cecal and faecal microbiota using rRNA-targeted probes. They found that *Bacteroides* and *Clostridium* groups (including, e.g. *Eubacterium* and *Ruminococcus*) represented 44% of the faecal bacterial rRNA, but only 13% of cecal bacterial rRNA.

2.2. Development of intestinal microbiota

Microbial colonisation of the intestines begins immediately after birth (Benno and Mitsuoka, 1986; Grönlund et al., 1999, 2000). The maternal intestinal microbiota is a source of bacteria colonising the intestine of a newborn. Colonisation is also determined by contact with the surroundings. At this stage, the most common bacterial strains are facultative anaerobes such as enterobacteria, coliforms and lactobacilli (Benno and Mitsuoka, 1986). Bifidobacteria are among the predominant culturable anaerobic bacteria in the intestinal microbiota from early infancy until the old age. However, bifidobacterial numbers decrease along with old age and the species/strain composition changes from infancy to adult years and old age (Benno and Mitsuoka, 1986; He et al., 2000, 2001; Ouwehand et al., 2001).

Normally, the first microbes colonising the intestinal tract of a newborn will be derived from the mother, and these microbes have a strong stimulatory effect for both the normal development of the microbiota and the maturation of the gut-associated lymphoid tissue. These effects are less apparent in caesarian born infants who are colonised by microbes from the hospital environment (Grönlund et al., 1999, 2000). Diet can have a major effect on the gut microbiota activities. In infants it is thought that those who are breast-fed have a natural predominance of bifidobacteria, and also specific strains of bifidobacteria. The formula-fed infants have a microbiota that is more complex and similar to the adults' microbiota, containing bifidobacteria, enterobacteria, lactobacilli, bacteroides, clostridia and enterococci (Benno and Mitsuoka, 1986; Kleessen et al., 1995). After weaning, the composition of the microbiota resembles that of the adult (Edwards, 1993).

2.3. Aberrations in intestinal microbiota

Altered gut microecology, reported in many gut-related inflammatory diseases, is clearly a common phenomenon. Inflammation is accompanied by imbalances in the intestinal microbiota. When the healthy host–microbe interaction is disturbed, an immune response can be induced by resident bacteria (Salminen et al., 1995). Duchmann et al. (1995) have previously demonstrated that healthy individuals are tolerant to their own microbiota, and that such tolerance is disturbed in patients with inflammatory bowel disease. Altered gut microbiota is reported in patients with rheumatoid arthritis (Malin et al., 1996) and allergic disease (Björkstén et al., 1999, 2001; Kalliomäki et al., 2001a; Kirjavainen et al., 2002), indicating that the normal gut microbiota constitutes an ecosystem responding to and regulating inflammation both in the gut and elsewhere in the body.

2.4. Indigenous bacteria as probiotics and their role in the management of GI-tract microbiota

The indigenous bacteria may be grouped either as potentially pathogenic or as health promoting (Salminen et al., 1998a). The strains with beneficial properties include among others bifidobacteria and lactobacilli, which are also among the predominant culturable microbes in healthy infants (He et al., 2001). Normalisation of the indigenous microbiota by specific strains of the healthy gut microbiota forms the basis of probiotic therapy (Salminen et al., 1998a) (Fig. 1). Oral introduction of probiotics may affect a decrease of abnormally high intestinal permeability and altered gut microecology, improving the intestine's immunological barrier functions and alleviating the intestinal inflammatory response (Fig. 2). The targets for probiotic therapy are thus identified as clinical conditions with impaired mucosal barrier function, particularly manifested by infectious and inflammatory disease (Fig. 2). Selection and use of probiotics is based on these factors, and criteria for

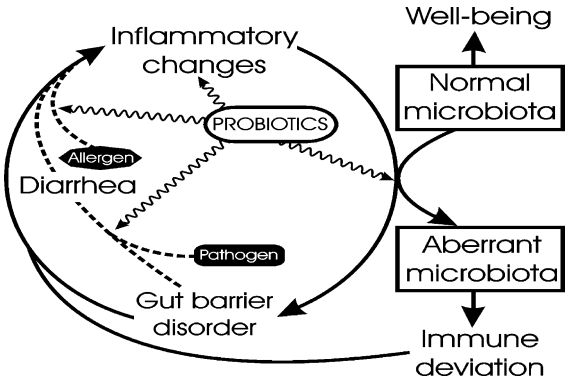

Fig. 2. Targets for probiotic intervetion in gut-associated dysfunctions.

probiotic selection have been chosen to counteract such clinical conditions (Salminen et al., 1998a; Isolauri et al., 2002). The most common indigenous bacteria used as probiotics are represented by bifidobacteria and lactobacilli, which have been attributed with beneficial aspects such as modification of the intestinal microbiota and stimulation of the immune response, thereby promoting non-specific host resistance to microbial pathogens (Salminen et al., 1998a; Lee et al., 2000; Pathmakanthan et al., 2000; Saarela et al., 2000).

3. Health-effects of probiotics

When assessing the health-promoting effects of probiotics, it is important to understand that all probiotic strains are different and their identification and characteristics should be well defined. Thus, studies on even closely related strains cannot be extrapolated without great caution. The assessment of the health-promoting potential of probiotics should be based on valid scientific hypothesis and studies supporting the hypothesis. Knowledge of the mechanisms is an important factor, complemented with target functions and biomarkers that are accepted as relevant to the state of health and well-being or reduction of risk of disease. The hypothesis can be supported by studies carried out in vitro using cell culture models or in vivo using animal models. However, the most important studies are carefully monitored clinical studies in human subjects. Studies on human subjects should be conducted preferably by at least two independent research groups in different locations (de Roos and Katan, 2000; Salminen et al., 1998a; Mattila-Sandholm et al., 1999; Pathmakanthan et al., 2000).

There are several other disorders and diseases, not discussed here, where probiotic nutritional management may have potential. These include mucosal vaccines and immunomodulation, infection control and eradication of multidrug-resistant microbes, treatment of candidal vaginitis, prevention of transmission of AIDS and sexually transmitted diseases, cholestrol and blood pressure lowering, and antimutagenic/anticarcinogenic activity (Alvarez-Olmos and Oberhelman, 2001; Isolauri et al., 2001; Kopp-Hoolihan, 2001; Kaur et al., 2002) (Fig. 1).

3.1. Probiotics and acute infections

The best-documented, most clearly reported evidence of the health benefits of specific probiotics concerns the treatment of viral diarrhoeal disorders in children. *Lactobacillus rhamnosus* GG has consistently reduced the duration of rotavirus-associated diarrhoea in randomised, controlled trials (Isolauri et al., 1994; Raza et al., 1995; Guarino et al., 1997; Guandalini et al., 2000). Trials with other specific probiotic strains (including *Lactobacillus reuteri*, *Bifidobacterium lactis* Bb-12 and *Saccharomyces boulardii*) have also shortened the duration and decreased the symptoms associated with acute viral diarrhoea (Chapoy, 1985; Saavedra et al., 1994; Majamaa et al., 1995; Shornikova et al., 1997a,b; Shu et al., 2001). Recent studies have also shown positive effects of probiotics in diarrhoeal diseases in children of day-care centres, although the difference has been small (Hatakka et al., 2001; Juntunen et al., 2001). Regular supplementation with probiotics may be effective in the prevention of acute diarrhoea in children, as was shown in a placebo-controlled trial of undernourished Peruvian children (Oberhelman et al., 1999). *L. rhamnosus* GG and *S. boulardii* have also proved effective in reducing antibiotic-associated diarrhoea and to treat *Clostridium difficile* diarrhoea (Surawicz et al., 1989; Buts et al., 1993; Siitonen et al., 1990; Arvola et al., 1999b).

L. rhamnosus GG, *S. boulardii*, and *Streptococcus thermophilus* were effective in preventing traveller's diarrhoea in some, but not in all subjects, in large-scale, placebo-controlled trials (Oksanen et al., 1990; Kollaritsch et al., 1993; Scarpignato and Rampal, 1995; Hilton et al., 1997). In yet another well-designed and controlled study, probiotic supplementation did no have any effect on the traveller's diarrhoea among British soldiers in Belize (Katelaris et al., 1995). The variation in geographic regions, pathogenic agents, and probiotic strains studied complicates data interpretation with respect to the effect of probiotics on traveller's diarrhoea. Further studies are clearly needed to verify this effect in different populations and travel destinations.

3.2. Probiotics and chronic disorders and diseases

Chronic diseases are an attractive target for probiotic risk reduction and nutritional management for

several reasons. Probiotics can safely be consumed as a part of the every-day diet and side-effects of probiotic consumption have been extremely rare even following extended periods of high dose consumption. Since a probiotic added to foods is not a medicine, the threshold for using these products both for the reduction of the risk of disease and for the nutritional management of disease relapse is low. For obvious reasons, the chronic diseases targeted for probiotic intervention have been gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), where the gut microbiota aberrancy and/or abnormal response to normal gut microbiota are suspected to have a role in the onset of the disease. Other chronic disorders where gut microbes are likely to play a role, and where probiotics could therefore be applied, include rheumatoid arthritis and atopic diseases.

3.2.1. Irritable bowel syndrome (IBS)

IBS is defined as a functional bowel disorder in which abdominal pain is associated with a change in bowel habit with features of disordered defecation and distention. It is the most common disorder diagnosed by gastroenterologists and the overall prevalence rate is about 10% in industrialised countries (Camilleri, 2001). Several mechanisms are proposed to play a role in IBS. These include psychosocial factors, altered bowel motility, heightened function of the intestine, imbalance in neurotransmitters, and infection (Horwitz and Fisher, 2001). It has been suggested that symptoms of IBS could be (at least partly) attributable to disturbed intestinal microbiota (Balsari et al., 1982; Bradley et al., 1987; Borody et al., 1989; King et al., 1998). Increased colonic gas production, common in IBS subjects, has been explained by an abnormal colonic fermentation (King et al., 1998; Nobaek et al., 2000) and by excessive swallowed air (Haderstorfer et al., 1989).

The results obtained in studies investigating the role of intestinal microbiota and colonic fermentation in IBS are contradictory and therefore the question of whether a microbial imbalance exists in IBS subjects remains open. Despite this controversy, several studies have assessed the effect of bacterial treatment on IBS symptoms. There are a few early reports (Rafsky and Rafsky, 1955; Winkelstein, 1956; Beck and Necheles, 1961) that suggest that live *Lactobacillus acidophilus* can be useful in the therapy of functional intestinal disorders including cases of IBS. Gade and Thorn (1989) compared the effect of freeze-dried culture of *Enterococcus faecium* and placebo in 54 IBS subjects. After 4 weeks of daily consumption of *E. faecium* (the daily dose of the bacterium was not given) and placebo, 81% of the *E. faecium*-group and 41% of the placebo-group subjects had improved according to physicians' assessments. Halpern et al. (1996) investigated the effect of heat-killed *L. acidophilus* (daily dose 2×10^{10} CFU) on the symptoms of IBS in a randomised, double-blind, cross-over (2×4 weeks feeding period with 2 weeks washout in between) trial in 18 subjects. They demonstrated that 9/18 subjects in the *L. acidophilus*-group and 1/18 subject in the placebo-group improved when the overall effect on six clinical criteria was assessed.

Nobaek et al. (2000) included 60 IBS subjects in their study where the subjects were randomised into two groups, one receiving 400 ml of a rose-hip drink containing *Lactobacillus plantarum* DSM 9843 (daily dose 2×10^{10} CFU) and the other plain rose-hip drink for 4 weeks. The results indicate that flatulence was rapidly reduced in the *L. plantarum*-group, whereas abdominal pain was reduced in both groups. At the 12-month follow-up subjects who had been in the *L. plantarum*-group maintained a better overall GI function. In another study performed with *L. plantarum* (strain 299 V, same as DSM 9843, daily dose 2×10^{10} CFU for 4 weeks) an improvement in symptoms of IBS was also noted in the test group of 20 subjects (Niedzielin et al., 2001). O'Sullivan and O'Morain (2000) studied the efficacy of *L. rhamnosus* GG supplementation (daily dose 10^{10} CFU) in improving symptoms of IBS. In the 19 subjects completing the study, no significant differences were found in *L. rhamnosus* GG and placebo groups. However, since the number of subjects participating into the study was small, no conclusions on the usefulness of *L. rhamnosus* GG in the treatment of IBS can be drawn yet.

In addition to single bacterial strain treatments, the effect of bacterial mixtures on IBS has also been tested. In a preliminary report by Andrews and Borody (1993), a liquid infusion of a mixture of 18 bacteria (specifications not given) into the caecum of IBS subjects improved the symptoms in 25 of 33 patients. In the study of Brigidi et al. (2001), the efficacy of bacterial mixture VSL-3 (same as VSL#3

below), containing four *Lactobacillus* strains, three *Bifidobacterium* strains and a *S. thermophilus* strain, was tested on 10 subjects with either IBS or functional diarrhoea (FD). In this preliminary study (no control group was included), both IBS and FD subjects reported a clear clinical improvement after consumption of the probiotic mixture (daily dose 9×10^{11} CFU for 20 days).

Although the results of the above studies suggest that microbial imbalance may play a role in IBS, and that a bacterial/probiotic treatment may be effective in reducing IBS symptoms, the comparison and interpretation of results is difficult. Since IBS is diagnosed on the basis of a series of symptom criteria and limited evaluation to exclude organic disease, IBS subjects are bound to form a heterogeneous group. So-called Rome criteria are often applied to define IBS (Camilleri, 2001), but these criteria are not uniformly used. Furthermore, other disorders like lactose intolerance, some chronic intestinal infections, and small intestinal bacterial overgrowth can have symptoms indistinguishable from IBS. Therefore, the hypothesis and preliminary indications on probiotic efficacy in alleviating IBS symptoms warrants further studies in large, well-defined patient populations.

3.2.2. Inflammatory bowel disease (IBD)

IBD refers to disorders of unknown cause that are characterised by chronic and recurrent intestinal infection. Ulcerative colitis (a relapsing inflammatory disorder of the colon), Crohn's disease (a chronic IBD occurring anywhere from the mouth to the anus), and pouchitis (a non-specific inflammation of the ileal reservoir) are generally included in IBD. The mechanisms responsible for onset of IBD remain unknown, but it is assumed that IBD results from abnormal host response towards normal GI microbiota or from a defective mucosal barrier (Marteau et al., 2001a). In Crohn's disease and ulcerative colitis, an imbalance in the GI microbiota has also been suggested (Kallinowski et al., 1998; Dunne et al., 1999; Kennedy et al., 2000).

Although several promising animal studies have been performed to study the efficacy of probiotic bacteria in IBD (Schultz and Sartor, 2000), human studies are fairly scarce. Gionchetti and et al. (2000) studied the efficacy of the probiotic preparation VSL#3 (daily dose 3×10^{12} CFU for 9 months) in the maintenance of remission in chronic pouchitis. In the placebo group, all 20 subjects had relapses during the study period. In the probiotic group, the corresponding figure was 3/20, which suggests that oral administration of the probiotic mixture was effective in preventing flare-ups of chronic pouchitis.

Malin et al. (1996) assessed the immunostimulatory effect of *L. rhamnosus* GG (daily dose 2×10^{10} CFU for 10 days) on 14 children with Crohn's disease. *L. rhamnosus* GG was shown to stimulate the gut IgA immune response and the authors concluded that *L. rhamnosus* GG could have potential in promoting the gut immunological barrier in Crohn's disease. Gupta et al. (2000) continued the studies on *L. rhamnosus* GG efficacy in Crohn's disease. In their open-label pilot study *L. rhamnosus* GG (daily dose 2×10^{10} CFU) was given for 6 months to four children with Crohn's disease. The findings of the pilot study suggested that *L. rhamnosus* GG might improve gut barrier function and clinical status of the Crohn's disease patients.

Two clinical trials on the efficacy of non-pathogenic *Escherichia coli* strain Nissle 1917 versus mesalazine (an aminosalicylate) in the treatment of ulcerative colitis have been performed. Kruis et al. (1997) compared the efficacy of *E. coli* strain Nissle 1917 and a standard treatment (mesalazine) in maintaining remission in 120 ulcerative colitis patients. Patients received either mesalazine (500-mg t.d.s.) or *E. coli* (daily dose 5×10^{10} CFU) for 12 weeks. The results indicated that the *E. coli* preparation was as effective as mesalazine in maintaining remission during the studied period. Rembacken et al. (1999) investigated the efficacy of the *E. coli* treatment in preventing relapse of ulcerative colitis during a longer period of time. Patients were randomised to receive either mesalazine ($N=59$, initial daily dose 2.4 g) or *E. coli* ($N=57$, initial daily dose 10^{11} CFU) concomitantly with 1-week gentamicin therapy (daily dose 240 mg). Patients continued *E. coli* or mesalazine treatment until they reached remission (max. 12 weeks). Thereafter the subjects entered a maximum 12 months follow-up phase ($N=44$ in the mesalazine group, $N=39$ in the *E. coli*-group) during which the doses of both *E. coli* and mesalazine were reduced to half. No significant differences were detected between the two groups regarding the time needed to reach remission, duration of remission or number of subjects

relapsing. The results suggest that *E. coli* treatment was as effective as mesalazine in maintaining remission of ulcerative colitis. The results of Kruis et al. (1997) and Rembacken et al. (1999) are in agreement with each other and suggest that *E. coli* strain Nissle 1917 administration forms an alternative nutritional strategy for standard medication in maintaining remission of ulcerative colitis.

Venturi et al. (1999) proposed a different approach and they studied the clinical efficacy of a probiotic mixture (preparation VSL#3) in ulcerative colitis subjects in remission. Twenty ulcerative colitis patients, intolerant or allergic to 5-aminosalicylic acid, consumed a VSL#3 preparation (bacterial daily dose 3×10^{12} CFU) for 12 months. The results, which must be interpreted with caution since no control group was included in the trial, suggested that VSL#3 might be useful in maintaining the remission in ulcerative colitis patients.

3.2.3. Arthritis

In Crohn's disease and ulcerative colitis, the bowel inflammation is complicated by joint inflammation in about 20% of the patients. Furthermore, bowel infection by enteropathogens such as *Salmonella* and *Yersinia* can provoke joint inflammation in patients. These facts support the theory that intestinal bacteria play a role in the etiology of rheumatoid arthritis (Hazenberg et al., 1992). Possible mechanisms include altered bowel anatomy, autoimmunity due to molecular mimicry, altered bowel permeability, and toxin mediated synovitis (Phillips, 1989). Arthropathic properties of intestinal bacteria have been widely studied in animal models (for a review, see Hazenberg et al., 1992). However, in animal (rat) studies, the bacteria were most often introduced by intraperitoneal injection, which is an extreme situation and hardly reflects the situation in humans. By using intraperitoneal injection in a rat model, several bacterial species of the normal GI-tract microbiota, including lactobacilli and bifidobacteria, have been shown to induce arthritis (Severijnen et al., 1989; Simelyte et al., 2000), but animal studies have to be interpreted with extreme caution. On the other hand, in rheumatoid arthritis patients on a vegan diet rich in lactobacilli, a clear improvement in disease has been observed (Peltonen et al., 1997; Nenonen et al., 1998). Despite the fact that arthritis patients seem to benefit from a lactobacilli-rich diet, no human trials on the efficacy of probiotics in the nutritional management of the disease have been performed.

3.2.4. Food allergies and atopic disease

Several recent studies have suggested a role for gut-colonising bacteria in preventing and treating manifestations of food allergy and atopic disease, including atopic eczema, asthma, and other allergies. It has been proposed that children developing allergy may have an aberrant gut microbiota (Kalliomäki et al., 2001a). Documented beneficial effects of probiotics on allergy development have primarily resulted from studies on infants and children (Majamaa and Isolauri, 1997; Pessi et al., 2000; Kalliomäki et al., 2001b). In a placebo-controlled study of high-risk infants, supplementation of both mothers and infants with *L. rhamnosus* GG significantly reduced the incidence of atopic disease at 2 years of age (Kalliomäki et al., 2001b). In other studies, supplementation of hydrolysed whey formula with *L. rhamnosus* GG and *B. lactis* Bb-12 significantly alleviated symptoms of atopic eczema in infants with milk hypersensitivity (Majamaa and Isolauri, 1997; Isolauri et al., 2000).

As mentioned, it has been reported that infants developing atopic diseases may have an aberrant microbiota as compared to children who remain healthy, an imbalance that probiotics could potentially correct. Although the mechanism of action of probiotics in preventing allergy is only beginning to be elucidated, the effects are likely to be mediated by adhesion to intestinal mucus and mucosal surfaces (He et al., 2001; Ouwehand et al., 2001) and generation of anti-inflammatory cytokines (Cross and Gill, 2001). It has been hypothesised that prevention of early atopic disease in children may reduce the risk of developing food allergy and asthma later in life. Evidence that probiotics may reduce allergic symptoms in existing atopic disease is also accumulating for specific strains and their use in early infancy. These have been documented by Majamaa and Isolauri (1997) and Isolauri et al. (2000) and are also in principle supported by the recent work on prevention of atopic diseases (Kalliomäki et al., 2001b). Although probiotics may reinforce endogenous gut microbiota, supplementation of common yoghurt with several strains of dairy starter lactic acid bacteria failed to result in the alleviation of clinical symptoms

in adult subjects with asthma and allergic rhinitis (Wheeler et al., 1997a,b).

4. Consumers and perceived health benefits of probiotics

The scientific evidence on the role of probiotic bacteria in promoting our well-being has increased in recent years. When probiotics are added to food products, the success of these products will be determined by consumers' willingness to buy and eat them. The crucial question for the acceptance is how consumers perceive the benefits these new products will provide. Some of the effects, such as preventing traveller's diarrhoea or other temporary gut disorders, are beneficial to a large group of people, but many effects benefit a well-defined target group. For patients with IBS or IBD, probiotics can provide clear reduction or even prevention of symptoms. Benefits that appeal to people with no apparent problems are probably different from those that appeal to patients who suffer from chronic disorders.

For consumers, the idea of functional foods, i.e. products containing probiotics targeted for improving special physiological functions, represents a new kind of health promotion. Instead of thinking of the overall diet, consumers are promised health benefits through using single products. The new kinds of healthiness in foods appear to respond to the needs of different people than the idea of a nutritionally adequate diet. The most enthusiastic users of functional foods are believed to be those middle aged women who already have healthy diets and little need to improve their diets (Sloan, 2000). However, the general health interest (Roininen et al., 1999), which measure people's willingness to comply with nutritional recommendations, has not been strongly linked with the willingness to use foods with special functional claims in Finnish studies (Lähteenmäki et al., 2001). The difference was also demonstrated in a study that explored consumers' impressions of functional food users (Saher et al., 2001). Generally, buyers of foods that had a healthy image were regarded as disciplined, whereas consumers of functional foods were perceived to be innovative.

Developing probiotic foods that promise new benefits to the consumer also introduce the novelty aspect into the product, which has an impact on consumer acceptance. Consumers tend to be suspicious towards new foods (e.g. Lähteenmäki and Arvola, 2001) as the novelty also means uncertainty and thus risk. The benefits promised by these new food products will be weighed against the possible risks and uncertainty linked with them (Frewer et al., 1997). Furthermore, production of new probiotic foods often requires high technology solutions in manufacturing. Healthiness in many countries is linked with naturalness (Lappalainen et al., 1998). The technology required to produce these special functions may interfere with the perceived naturalness of the products and thereby cause suspicion in consumers' minds (Poulsen, 1999). The perception of benefits seems to be strongly linked with the product–claim interaction. A function that re-enforces the natural properties of a product is easier to accept than a function that is artificially implanted to the product or is in discordance with the previous image of the product. Therefore, adding probiotics into food products requires, in addition to technological considerations, thought concerning the beliefs consumers have about these products.

Most of the effects promised by modification of the gut flora take time and physiological effects are therefore not experienced directly. The key issue in foods with health-related claims is the credibility of the information (Grunert et al., 2000; Bech-Larsen et al., 2001). In some cases, physiological effects can directly influence our responses to foods (Rozin, 1982; Yeomans et al., 2000). However, comparable cases among so-called functional foods are not available. The reward value of a probiotic message relies on the perceived benefit conveyed by the message and our trust in it. We tend to give our attention to messages that are relevant to us and which are congruent with our earlier beliefs. Therefore, in order to motivate our choice, food-related messages have to fit into our existing beliefs. In an Irish study, the most interesting claims for the consumers were related to heart disease, cancer and bone health (Bogue and Ryan, 2000). Probiotics were not yet well known, although yoghurt was among the most frequently used functional foods.

The future acceptance of probiotic products depends on their ability to provide consumers the benefits that they promise at the present. This requires research that provides necessary additional

evidence to back up the health claims made in these products. There is also a challenge to present the knowledge produced by science in ways that are comprehensible for the consumer. Consumer thinking is based on dichotomic thinking and approximation (Lindeman, 1998), whereas scientific thinking uses probabilities and accepts a certain degree of uncertainty. For consumers questions tend to have yes or no answers, and even with deficient pieces of information consumers tend to build holistic rationales for themselves. In scientific thinking, the degree of certainty can be assessed and gaps in knowledge are acknowledged. Consumers' trust in the information depends on the source and content of the message. Producers of probiotic foods have to be careful in ensuring that this trust exists and is gradually enhanced.

Although important, healthiness is only one reason behind food choices and other factors often decide choices. Good taste and other sensory qualities can be experienced directly and they are known to be the key factors in repeated choices (e.g. Arvola et al., 1999a; Tepper and Trail, 1998). Even with foods aimed for health, the sensory qualities have to convince consumers.

There exists a plethora of questions that remain to be answered in terms of consumer awareness and the best methods to convey health messages. How to communicate the gut health messages effectively to consumers of varying age and cultural background remains one of the key issues. Furthermore, how to create and sustain trust between manufacturers and consumers, so that messages will be received rather than ignored. Scientists and manufactures invest a lot of effort into establishing the functional properties of different probiotic strains and products. However, whether consumers are aware of these differences or whether they believe that the benefits of various strains are just the same has not been answered. Food applications that bring relief of diseases may combine the idea of food and medicine in a way that is difficult for consumers to accept and finding a place for these kind of foods in the regular food system may be difficult. These questions must be addressed to ensure that scientific achievements are translated into appropriate products which will be accepted and consumed and thus have a role in improving human health.

5. Development of efficacious functional foods for intestinal health

A number of steps are essential in the development of efficacious probiotic and prebiotic functional foods (Fig. 3). A sample scheme has been develop for functional foods in the treatment of food allergy during the preceding framework programme (Isolauri et al., 1999). A prerequisite for mechanistic studies of probiotic action is an understanding of the composition and activity of the intestinal microbiota as well as interactions with the host in both healthy and diseased individuals. High-throughput molecular methods are required to examine the intestinal microbiota and to track the location and activity of probiotic strains in the intestinal tract. An understanding of the mechanisms by which probiotics exert beneficial effects on human health allows selection of strains with appropriate traits and hypothesis-driven clinical studies. The safety of new strains must be demonstrated before they are used in human clinical trials. An important area of research is technologies to maximise the stability of functional traits during manufacture, formulation, storage and in the intestinal tract. Additionally, the efficacy of products may be enhanced by exploiting synergistic interactions between functional ingredients, as is potentially the case with synbiotics. Finally, an understanding of the most appropriate methods to communicate the benefits of the functional foods to consumers and the influence of health messages on consumer choice is essential to ensure that such products are appropriately applied and targeted to benefit specific populations.

A concerted research effort is underway to study the application of probiotics and prebiotics for improved human health through the European Commission's 5th Framework Programme. Eight pan-European collaborative projects covering almost all of the major areas of research are currently underway, running from 2001 through to 2004 (Fig. 3, Table 2) (Mattila-Sandholm et al., 2002).

5.1. Research developments in studies on GI-tract microbiota

Advanced automated molecular methods for monitoring human gut microbiota composition and gene expression will be developed in "Development and application of high throughput molecular methods for

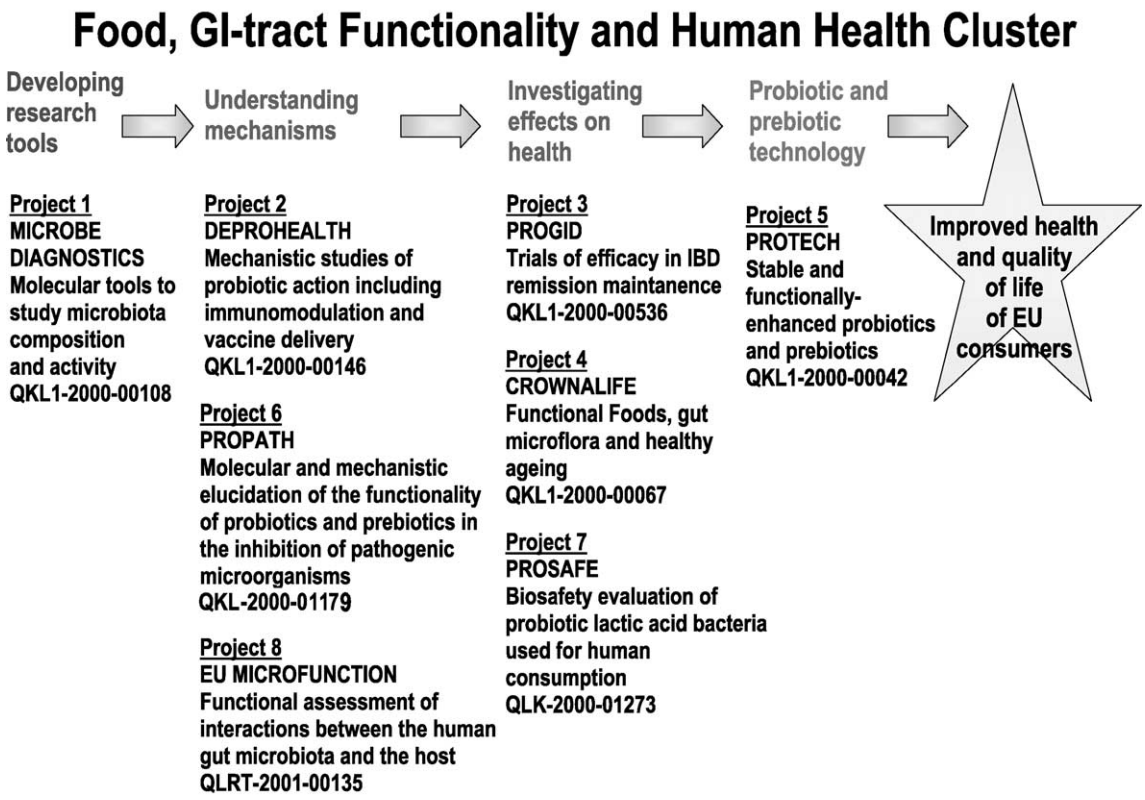

Fig. 3. The outline of the PROEUHEALTH cluster.

studying the human intestinal microbiota in relation to diet and health''. This project aims to develop and apply molecular tools to identify links between intestinal dysfunctions and intestinal bacteria, and to provide a greater understanding of mechanisms underlying the relationships between diet, life style, intestinal bacteria and optimal health.

5.2. Research developments in studies on mechanisms of probiotic functionality

The project titled ''Molecular analysis and mechanistic elucidation of the functionality of probiotics and prebiotics in the inhibition of pathogenic micro-organisms to combat gastrointestinal disorders and to improve human health'' focuses on molecular mechanisms of inhibition of Gram-negative, diarrhoeagenic bacteria as well as *Helicobacter pylori* by probiotics, and the enhancement of inhibition provided by probiotics through the use of prebiotics.

Central to the elucidation of probiotic mechanisms is a thorough understanding of how bacteria within the intestinal tract interact with the host. The project ''Functional assessment of interactions between the human gut microbiota and the host'' centres on elucidation of molecular mechanisms of cross-communication between intestinal bacteria and the host. The prophylactic benefits of probiotics, prebiotics and synbiotics against pathogenic bacterial translocation are being examined.

''Probiotic strains with designed health properties'', will focus on mechanistic studies. Molecular mechanisms affecting immunomodulation by probiotic lactobacilli will be examined using specifically designed bacterial strains. This will provide a better understanding of the molecular factors affecting immunomodulation and immunogenicity, enabling the selection of probiotic strains with enhanced protective or therapeutic effects. This knowledge will also be used to design and assess new probiotics as

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vaccine delivery vehicles. Inflammations such as IBD and intestinal pathogens such as rotavirus and *H. pylori* will be targeted in this project.

5.3. Research developments in studies on probiotic health-effects

One advantage of pre- and probiotics as functional foods is that they can be optimised and targeted towards specific population or risk groups, ‘‘Functional foods, gut microflora and healthy ageing’’ aims to develop and test pre- and probiotics for elderly populations. Within this project the effect of ageing on the composition and activity of the intestinal microbiota will be investigated in order to develop strategies to protect against degenerative intestinal diseases and reduce susceptibility to infection within this population group.

The results on the efficacy of probiotics in IBD are very promising although publications on human clinical trials are so far scarce. To address this, the project ‘‘Probiotics and gastrointestinal disorders—controlled trials of European Union patients’’ will assess the efficacy of two specially selected probiotics in alleviating the effects of IBD in long-term human clinical trials. The project will also explore the role of the human intestinal microbiota in these diseases at a mechanistic level.

5.4. Research developments in studies on probiotic safety

Safety assessment is an essential phase in the development of any new pre- or probiotic functional food (Salminen et al., 1998b). The safety record of probiotics is good, and lactobacilli and bifidobacteria are generally regarded as safe. The demonstration EU programme on probiotics established a list of safety criteria for probiotic foods illustrated in Table 3. All probiotic strains must be evaluated for their safety before being used in human clinical studies and in functional food products. Conventional toxicology and safety evaluation alone is of limited value in the safety evaluation of probiotic bacteria. Instead, a multidisciplinary approach is necessary involving contributions from pathologists, geneticists, toxicologists, immunologists, gastroenterologists and microbiologists. The project entitled ‘‘Biosafety evaluation of probiotic lactic acid bacteria used for human consumption’’ is further examining the issues in the safety of probiotic organisms including presence and

Table 3
Recommendations for safety of probiotic cultures and foods (the Probdemo approach; Salminen et al., 1998b)

(1) The producer that markets the food has the ultimate responsibility for supplying a safe food. Probiotic foods should be as safe as other foods.
(2) When the probiotic food turns out to be a novel food, it hence will be subject to the appropriate legal approval (EU directive for novel foods).
(3) When a strain has a long history of safe use, it will be safe as a probiotic strain and will not result in a novel food.
(4) The best test for food safety is a well-documented history of safe human consumption. Thus, when a strain belongs to a species for which no strains are known that are pathogenic and for which other strains have been described that have a long history of safe use, it is likely to be safe as a probiotic strain and will not result in a novel food.
(5) When a strain belongs to a species for which no pathogenic strains are known but which do not have a history of safe use, it may be safe as a probiotic strain but will result in a novel food and hence should be treated as such.
(6) When a new strain belongs to a species for which strains are known that are pathogenic, it will result in a novel food.
(7) Proper state of the art taxonomy is required to describe a probiotic strain. Today it includes DNA–DNA hybridization and rRNA sequence determination. This reasoning specifically applies to mutants of a probiotic strain.
(8) In line with recommendation (1), strains that carry transferable antibiotic resistance genes, i.e. genes encoding proteins that inactivate antibiotics should not be marketed.
(9) Strains that have not been properly taxonomically described using the approaches as indicated above under (7) should not be marketed. Strains should also be deposited in an internationally recognised culture collection.

horizontal transfer of antibiotic resistance genes, detection of virulence factors, evaluation of adverse immunological effects, and a study of the survival, colonisation and genetic stability of probiotics in the human gut. There has been considerable debate on what constitutes appropriate safety testing for new probiotic strains proposed for human consumption. The project will provide recommendations for standardised pre-marketing safety testing and post-marketing surveillance of probiotics.

5.5. Research developments in studies on probiotic technology

Maintaining the viability, stability, and functionality of probiotics during processing, formulation and storage is essential to delivering the health benefits of these ingredients to consumers. ‘‘Nutritional enhancement of probiotics and prebiotics: Technology aspects on microbial viability, stability, functionality and on prebiotic function’’ will explore the effects of processing on probiotics and develop optimal process and formulation technologies to maintain the stability and functionality of probiotics. New processing techniques will be applied to the development of functionally enhanced prebiotics and synbiotic combinations.

6. Conclusions

Microbes with health impact will remain an important functional ingredient also in the future. New strains will be identified and foods will be developed to fulfil the needs of specific consumer groups. Increased understanding of interactions between gut microbiota, diet and the host will open up new possibilities of producing new ingredients for nutritionally optimised foods, which promote consumer health through microbial activities in the gut.

The future scientific and technological research needs include:

- to study the mechanisms of action of microbes and their health effects in the GI-tract, and the development of diagnostic procedures and biomarkers for their assessment;
- to examine the effects of food-derived bioactive compounds on GI-diseases, GI-infections, and allergies;
- to develop new therapeutic and prophylactic treatments for different patient and population groups;
- to achieve molecular insight into the immune modulation by bacteria with health-effects;
- to elucidate the role of colon microbiota in the conversion of bioactive compounds;
- to study the effects of the metabolites on the colon epithelium or systemically after absorption;
- to evaluate the safety of functional ingredients;
- to ensure the stability of microbes with health effects and of their bioactive compounds, also in new types of food applications by developing feasible technologies;
- to address consumer expectations about health foods in different consumer groups.

References

Alvarez-Olmos, M.I., Oberhelman, R.A., 2001. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. Clinical Infectious Diseases 32, 1567–1576.

Andrews, P.J., Borody, T.J., 1993. Putting back the bugs: bacterial treatment relieves chronic constipation and symptoms of irritable bowel disease. The Medical Journal of Australia 159, 633–634.

Arvola, A., Lähteenmäki, L., Tuorila, H., 1999a. Predicting the intent to purchase unfamiliar and familiar cheeses: the effects of attitudes, expected liking and food neophobia. Appetite 32, 113–126.

Arvola, T., Laiho, K., Torkkeli, S., Mykkänen, H., Salminen, S., Maunula, L., Isolauri, E., 1999b. Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 104, e64.

Balsari, A., Ceccarelli, A., Dubini, F., Fesce, E., Poli, G., 1982. The fecal microbial population in the irritable bowel syndrome. Microbiologica 5, 185–194.

Bech-Larsen, T., Grunert, K.G., Poulsen, J.B., 2001. The acceptance of functional foods in Denmark, Finland and the United States. MAPP Working paper no. 73. The Aarhus School of Business.

Beck, C., Necheles, H., 1961. Beneficial effects of administration of *Lactobacillus acidophilus* in diarrheal and other intestinal disorders. American Journal of Gastroenterology 35, 522–526.

Benno, Y., Mitsuoka, T., 1986. Development of intestinal microflora in humans and animals. Bifidobacteria Microflora 5, 13–25.

Berg, R.D., 1996. The indigenous gastrointestinal microflora. Trends in Microbiology 4, 430–435.

Björkstén, B., Naaber, P., Sepp, E., Mikelsaar, M., 1999. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clinical and Experimental Allergy 29, 342–346.

Björkstén, B., Sepp, E., Julge, K., Voor, T., Mikelsaar, M., 2001. Allergy development and the intestinal microflora during the first year of life. Journal of Allergy and Clinical Immunology 108, 516–520.

Bogue, J., Ryan, M., 2000. Market-oriented new product development: Functional foods and the Irish consumer. Agribusiness Discussion paper No. 27. Department of Food Economics, University College, Cork, Ireland.

Borody, T.J., George, L., Andrewes, P., Brandl, S., Noonan, S., Cole, P., Hyland, L., Morgan, A., Maysey, J., Moore-Jones, D., 1989. Bowel-flora alteration: a potential cure for inflammatory bowel disease and irritable bowel syndrome? The Medical Journal of Australia 150, 604.

Bradley, H.K., Wyatt, G.M., Bayliss, C.E., Hunter, J.O., 1987. Instability in the faecal flora of a patient suffering from food related irritable bowel syndrome. Journal of Medical Microbiology 23, 29–32.

Brigidi, P., Vitali, B., Swennen, E., Bazzocchi, G., Matteuzzi, D., 2001. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. Research in Microbiology 152, 735–741.

Buts, J.P., Corthier, G., Delmee, M., 1993. *Saccharomyces boulardii* for *Clostridium difficile*-associated enteropathies in infants. Journal of Pediatric Gastroenterology and Nutrition 16, 419–425.

Camilleri, M., 2001. Management of the irritable bowel syndrome. Gastroenterology 120, 652–668.

Chapoy, P., 1985. Treatment of acute infantile diarrhea: controlled trial of *Saccharomyces boulardii* (article in French). Annals of Pediatrics (Paris) 32, 561–563.

Cross, M., Gill, H.S., 2001. Can immunoregulatory lactic acid bacteria be used as dietary supplement to limit allergies? International Archives of Allergy and Immunology 125, 112–119.

de Roos, N.M., Katan, M.B., 2000. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. American Journal of Clinical Nutrition 71, 405–411.

Duchmann, R., Kaiser, I., Hermann, E., Mayet, W., Ewe, K., Meyer zum Büschenfelde, K.H., 1995. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). Clinical and Experimental Immunology 1995, 448–455.

Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., Morrisey, D., Thornton, G., Fitzgerald, G., Daly, C., Kiely, B., Quigley, E.M.M., O'Sullivan, G., Shanahan, F., Collins, K., 1999. Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie van Leeuwenhoek 76, 279–292.

Edwards, C., 1993. Interactions between nutrition and intestinal microflora. Proceedings of the Nutrition Society 52, 375–382.

Finegold, S.M., Attebery, H.R., Sutter, V.L., 1974. Effect of diet on human fecal flora: comparison of Japanese and American diets. American Journal of Clinical Nutrition 27, 1456–1469.

Franks, A.H., Marmsen, H.J.M., Raangs, G.C., Jansen, G.J., Schut, F., Welling, G.W., 1998. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16 S rRNA-targeted oligonucleotide probes. Applied and Environmental Microbiology 64, 3336–3345.

Frewer, L.J., Howard, C., Hedderley, D., Shepherd, R., 1997. Consumer attitudes towards different food-processing technologies used in cheese production—the influence of consumer benefit. Food Quality and Preference 8, 271–280.

Fuller, R., 1989. Probiotics in man and animals. Journal of Applied Bacteriology 66, 365–378.

Gade, J., Thorn, P., 1989. Paraghurt for patients with irritable bowel syndrome. Scandinavian Journal of Primary Health Care 7, 23–26.

Gaskins, H.R., 1997. Immunological aspects of host/microbiota interactions at the intestinal epithelium. In: Mackie, R.I., White, B.A., Isaacson, R.E. (Eds.), Gastrointestinal Microbiology. International Thomson Publishing, New York, pp. 537–587.

Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125, 1401–1412.

Gionchetti, P., Rizzello, F., Venturi, A., Birigidi, P., Matteuzzi, D., Bazzocchi, G., Poggioli, G., Miglioli, M., Campieri, M., 2000. Oral bacteriotherapy as maintenance treatment in patients with

chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 119, 305–309.

Grönlund, M.M., Lehtonen, O.P., Eerola, E., Kero, P., 1999. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. Journal of Pediatric Gastroenterology and Nutrition 28, 19–25.

Grönlund, M.M., Arvilommi, H., Kero, P., Lehtonen, O.P., Isolauri, E., 2000. Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. Archives of Diseases in Children 83, F186–F192.

Grunert, K., Bech-Larsen, T., Bredahl, L., 2000. Three issues in consumer quality perception and acceptance of dairy products. International Dairy Journal 10, 575–584.

Guandalini, S., Pensabene, L., Zikri, M.A., Doas, J.A., Casali, L.G., Hoekstra, H., Kolacek, S., Massar, K., Micetic-Turk, D., Papadopoulou, A., de Sousa, J.S., Sandhu, B., Szajewska, H., Weizman, Z., 2000. *Lactobacillus* GG administered in oral solution to children with acute diarrhea: a multicenter European trial. Journal of Pediatric Gastroenterology and Nutrition 30, 54–60.

Guarino, A., Canani, R.B., Spagnuolo, M.I., Albano, F., Di Benedetto, L., 1997. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. Journal of Pediatric Gastroenterology and Nutrition 25, 516–519.

Gupta, P., Andrew, H., Kirschner, B.S., Guandalini, S., 2000. Is *Lactobacillus* GG helpful in children with Crohn's disease? Results of preliminary, open-label study. Journal of Pediatric Gastroenterology and Nutrition 31, 453–457.

Haderstorfer, B., Whitehead, W.E., Schuster, M.M., 1989. Intestinal gas production from bacterial fermentation of undigested carbohydrate in irritable bowel syndrome. American Journal of Gastroenterology 84, 375–378.

Halpern, G.M., Prindville, T., Blankenburg, M., Hsia, T., Gershwin, M.E., 1996. Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. American Journal of Gastroenterology 91, 1579–1585.

Hatakka, K., Savilahti, E., Pönkä, A., Meurman, J.H., Poussa, T., Näse, L., Saxelin, M., Korpela, R., 2001. Effect of long term consumption of probiotic milk on infections in children attending day care centers: double blind, randomised trial. British Medical Journal 322, 1327–1329.

Hazenberg, M.P., Klasen, I.S., Kool, J., Ruseler-van Embden, J.G.H., Severijnen, A., 1992. Are intestinal bacteria involved in the etiology of rheumatoid arthritis? Acta Pathologica, Microbiologica et Immunologica Scandinavica 100, 1–9.

He, F., Ouwehand, A., Isolauri, E., Hashimoto, H., Benno, Y., Salminen, S., 2000. Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants. FEMS Immunological and Medical Microbiology 1285, 43–47.

He, F., Ouwehand, A., Isolauri, E., Hosoda, M., Benno, Y., Salminen, S., 2001. Differences in composition and mucosal adhesion of bifidobacteria isolated from healthy adults and healthy seniors. Current Microbiology 43, 351–354.

Heasman, M., Mellentin, J., 2001. The Functional Foods Revolution—Healthy People, Healthy Profits? Earthscan Publications, Sterling, VA.

Hilliam, M.A., Young, J., 2000. Functional Food Markets, Innovation and Prospects: A Global Analysis. Leatherhead Publishing, Surrey, UK.

Hilton, E., Kolakowski, P., Singer, C., Smith, M., 1997. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travellers. Journal of Travel Medicine 4, 41–43.

Horwitz, B.J., Fisher, R.S., 2001. Current concepts: the irritable bowel syndrome. New England Journal of Medicine 344, 1846–1850.

Isolauri, E., Kaila, M., Mykkänen, H., Ling, W.H., Salminen, S., 1994. Oral bacteriotherapy for viral gastroenteritis. Digestive Diseases and Sciences 39, 2595–2600.

Isolauri, E., Salminen, S., Mattila-Sandholm, T., 1999. New functional foods in the treatment of food allergy. Annals of Medicine 31, 299–302.

Isolauri, E., Arvola, T., Sutas, Y., Moilanen, E., Salminen, S., 2000. Probiotics in the management of atopic excema. Clinical and Experimental Allergy 30, 1604–1610.

Isolauri, E., Sutas, Y., Kankaanpää, P., Arvilommi, H., Salminen, S., 2001. Probiotics: effects on immunity. American Journal of Clinical Nutrition 73, 444S–450S (Suppl.).

Isolauri, E., Rautava, S., Kalliomäki, M., Kirjavainen, P., Salminen, S., 2002. Role of probiotics in food hypersensitivity. Current Opinion in Allergy and Clinical Immunology 2, 263–271.

Juntunen, M., Kirjavainen, P.V., Ouwehand, A.C., Salminen, D.J., Isolauri, E., 2001. Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. Clinical and Diagnostic Laboratory Immunology 8, 293–296.

Kallinowski, F., Wassmer, A., Hofmann, M.A., Harmsen, D., Heesemann, J., Karch, H., Herfarth, C., Buhr, H.J., 1998. Prevalence of enteropathogenic bacteria in surgically treated chronic inflammatory bowel disease. Hepatogastroenterology 45, 1552–1558.

Kalliomäki, M., Kirjavainen, P., Eerola, E., Kero, P., Salminen, S., Isolauri, E., 2001a. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. Journal of Allergy and Clinical Immunology 107, 129–134.

Kalliomäki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P., Isolauri, E., 2001b. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 357, 1076–1079.

Katelaris, P.H., Salam, I., Farthing, M.J., 1995. Lactobacilli to prevent traveler's diarrhea? New England Journal of Medicine 333, 1360–1361.

Kaur, I.P., Chopra, K., Saini, A., 2002. Probiotics: potential pharmaceutic applications. European Journal of Pharmaceutical Sciences 15, 1–9.

Kennedy, R.J., Kirk, S.J., Gardiner, K.R., 2000. Promotion of favorable gut flora in inflammatory bowel disease. JPEN Journal of Parenteral and Enteral Nutrition 24, 189–195.

King, T.S., Elia, M., Hunter, J.O., 1998. Abnormal colonic fermentation in irritable bowel syndrome. Lancet 352, 1187–1189.

Kirjavainen, P.V., Apostolou, E., Arvola, T., Salminen, S.J., Gibson, G.R., Isolauri, E., 2001. Characterizing the composition of in-

testinal microflora as a prospective treatment target in infant allergic disease. FEMS Immunology and Medical Microbiology 32, 1–7.

Kirjavainen, P.V., Arvola, T., Salminen, S.J., Isolauri, E., 2002. Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? Gut 51, 51–55.

Kleessen, B., Bunke, H., Tovar, K., Noack, J., Sawatzki, G., 1995. Influence of two infant formulas and human milk on the development of the faecal flora in newborn infants. Acta Paediatrica 84, 1347–1356.

Kollaritsch, H., Holst, H., Grobara, P., Wiedermann, G., 1993. Prevention of traveller's diarrhea with *Saccharomyces boulardii*. Results of a placebo controlled double-blind study (article in German). Fortschritte der Medizin 111, 152–156.

Kopp-Hoolihan, L., 2001. Prophylactic and therapeutic uses of probiotics: a review. Journal of the American Dietetic Association 101, 229–238.

Kruis, W., Schütz, E., Fric, P., Fixa, P., Judmaiers, G., Stolte, M., 1997. Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. Alimentary Pharmacology and Therapeutics 11, 853–858.

Lähteenmäki, L., Arvola, A., 2001. Food neophobia and variety seeking—consumer fear or demand for new food products. In: Frewer, L.J., Risvik, E., Schifferstein, H. (Eds.), Food, People and Society. A European Perspective of Consumer Food Choices. Springer-Verlag, Berlin, pp. 161–175.

Lähteenmäki, L., Isoniemi, M., Urala, N., Ryhänen, E.-L., 2001. In: Ryhänen, E.-L., Salo, R. (Eds.), Uusien terveysvaikutteisten elintarvikkeiden hyväksyttävyys. MTT Julkaisuja Sarja A93. Elintarvikeklusterin tutkimusohjelman loppuraportti. MTT, pp. 61–66. (The acceptability of new functional foods; in Finnish).

Lappalainen, R., Kearney, J., Gibney, M., 1998. A pan-European survey of consumers attitudes to food, nutrition and health: an overview. Food Quality and Preference 9, 467–478.

Lee, Y.K., Lim, C.Y., Teng, W.L., Ouwehand, A.C., Tuomola, E.M., Salminen, S., 2000. Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. Applied and Environmental Microbiology 66, 3692–3697.

Lindeman, M., 1998. Motivation, cognition and pseudoscience. Scandinavian Journal of Psychology 39, 257–265.

Majamaa, H., Isolauri, E., 1997. Probiotics: a novel approach in the management of food allergy. Journal of Allergy and Clinical Immunology 99, 179–185.

Majamaa, H., Isolauri, E., Saxelin, M., Vesikari, T., 1995. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. Journal of Pediatric Gastroenterology and Nutrition 20, 333–338.

Malin, M., Suomalainen, H., Saxelin, M., Isolauri, E., 1996. Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus* GG. Annals of Nutrition and Metabolism 40, 137–145.

Marteau, P.R., de Vrese, M., Cellier, C.J., Schrezenmeier, J., 2001a. Protection from gastrointestinal diseases with the use of probiotics. American Journal of Clinical Nutrition 73, 430S–436S (Suppl.).

Marteau, P., Pochart, P., Dore, J., Bera-Mailler, C., Bernalier, A., Corthier, G., 2001b. Comparative study of bacterial groups within the human cecal and fecal microbiota. Applied and Environmental Microbiology 67, 4939–4942.

Mattila-Sandholm, T., Saarela, T., 2000. Probiotic functional foods. In: Gibson, G.R., Williams, C.M.F. (Eds.), Functional Foods—Concept to Product. Woodhead Publishing, Cambridge, England, pp. 287–313.

Mattila-Sandholm, T., Blum, S., Collins, J.K., Crittenden, R., de Vos, W., Dunne, C., Fondén, R., Grenov, G., Isolauri, E., Kiely, B., Marteau, P., Morelli, L., Ouwehand, A., Reniero, R., Saarela, M., Salminen, S., Saxelin, M., Schiffrin, E., Shanahan, F., Vaughan, E., von Wright, A., 1999. Probiotics: towards demonstrating efficacy. Trends in Food Science and Technology 10, 393–399.

Mattila-Sandholm, T., Blaut, M., Mercenier, A., de Vuyest, L., Gibson, G., Shahanan, F., Dore, J., Goossens, H., Knorr, D., Lähteenmäki, L., de Vos, W., 2002. The food, GI-tract functionality and human health cluster. Microbial Ecology in Health and Disease 14, 65–74.

Metchnikoff, E., 1907. The Prolongation of Life Heinemann, London.

Moore, W.E.C., Holdeman, L.V., 1974. Human fecal flora: the normal flora of 20 Japanese–Hawaiians. Applied Microbiology 27, 961–979.

Nenonen, M.T., Helve, T.A., Rauma, A.-L., Hänninen, O.O., 1998. Uncooked, lactobacilli-rich vegan food and rheumatoid arthritis. British Journal of Rheumatology 37, 274–281.

Niedzielin, K., Kordecki, H., Birkenfeld, B., 2001. A controlled double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. European Journal of Gastroenterolgy and Hepatology 13, 1143–1147.

Nobaek, S., Johansson, M.L., Molin, G., Ahrne, S., Jeppsson, B., 2000. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. American Journal of Gastroentrology 95, 1231–1238.

Oberhelman, R.A., Gilman, R.H., Sheen, P., Taylor, N.D., Black, R.E., Cabrera, L., Lescano, A.G., Meza, R., Madico, G., 1999. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. Journal of Pediatrics 134, 15–20.

Oksanen, P.J., Salminen, S., Saxelin, M., Hämäläinen, P., Ihantola-Vormisto, A., Muurasniemi-Isoviita, L., Nikkari, S., Oksanen, T., Pörsti, I., Salminen, E., Siitonen, S., Stuckey, H., Toppila, A., Vapaatalo, H., 1990. Prevention of travellers' diarrhoea by *Lactobacillus* GG. Annals of Medicine 22, 53–56.

O'Sullivan, M.A., O'Morain, C.A., 2000. Bacterial supplementation in the irritable bowel syndrome. A randomised double-blind placebo-controlled crossover study. Digestive and Liver Disease 32, 294–301.

Ouwehand, A.C., Isolauri, E., He, F., Hashimoto, H., Benno, Y., Salminen, S., 2001. Differences in *Bifidobacterium* flora composition in allergic and healthy infants. Journal of Allergy and Clinical Immunology 108 (1 Pt 1), 144–145.

Pathmakanthan, S., Meance, S., Edwards, C.A., 2000. Probiotics: a

review of human studies to date and methodological approaches. Microbial Ecology in Health and Disease 12 (Suppl. 2), 10–30.

Peltonen, R., Nenonen, M., Helve, T., Hänninen, O., Toivanen, P., Eerola, E., 1997. Faecal microbial flora and disease activity in rheumatoid arthritis during a vegan diet. British Journal of Rheumatology 36, 64–68.

Pessi, T., Sutas, Y., Hurme, M., Isolauri, E., 2000. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. Clinical and Experimental Allergy 30, 1804–1808.

Phillips, P.E., 1989. How do bacteria cause chronic arthritis? Journal of Rheumatology 16, 1017–1019.

Poulsen, J.B., 1999. Danish consumers' attitudes towards functional foods. MAPP Working paper no. 62. The Aarhus School of Business.

Rafsky, H.A., Rafsky, J.C., 1955. Clinical and bacteriological studies of a new *Lactobacillus acidophilus* concentrate in functional gastrointestinal disturbances. American Journal of Gastroenterology 24, 87.

Raza, S., Graham, S.M., Allen, S.J., Sultana, S., Cuevas, L., Hart, C.A., 1995. *Lactobacillus* GG promotes recovery from acute nonbloody diarrhea in Pakistan. The Pediatric Infectious Diseases Journal 14, 107–111.

Rembacken, B.J., Snelling, A.M., Hawkey, P.M., Chalmers, D.M., Axon, A.T.R., 1999. Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet 354, 635–639.

Roininen, K., Lähteenmäki, L., Tuorila, H., 1999. Quantification of consumer attitudes to health and hedonic characteristics of foods. Appetite 33, 71–88.

Rozin, P., 1982. Human food selection: the interaction of biology, culture and individual experience. In: Barker, L.M. (Ed.), The Psychobiology of Human Food Selection. Ellis Horwood, Chichester, pp. 225–254.

Saarela, M., Mogensen, G., Fonden, R., Mättö, J., Mattila-Sandholm, T., 2000. Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology 84, 197–215.

Saavedra, J.M., Bauman, N.A., Oung, I., Perman, J.A., Yolken, R.H., 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344, 1046–1049.

Saher, M., Arvola, A., Lindeman, M., Lähteenmäki, L., 2001. Impression of functional food consumers. Abstract, 4th Pangborn Sensory Science Symposium, Dijon, July 2001.

Salminen, S., Isolauri, E., Onnela, T., 1995. Gut flora in normal and disordered states. Chemotherapy 41, 5–15.

Salminen, S., Bouley, C., Boutron-Ruault, M.C., Cummings, J.H., Franck, A., Gibson, G.R., Isolauri, E., Moreau, M.C., Roberfroid, M., Rowland, I., 1998a. Functional food science and gastrointestinal physiology and function. British Journal of Nutrition 80, 147–171 (Suppl.).

Salminen, S., von Wright, A., Morelli, L., Marteau, P., Brassart, D., de Vos, W., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.-E., Mattila-Sandholm, T., 1998b. Demonstration of safety of probiotics—a review. International Journal of Food Microbiology 44 (1–2), 93–106.

Scarpignato, C., Rampal, P., 1995. Prevention and treatment of traveller's diarrhea: a clinical pharmacological approach. Chemotherapy 41 (Suppl. 1), 48–81.

Schultz, M., Sartor, R.B., 2000. Probiotics and inflammatory bowel diseases. The American Journal of Gastroenterology 95, S19–S21.

Severijnen, A.J., van Kleef, R., Hazenberg, M.P., van de Merwe, J.P., 1989. Cell wall fragments from major residents of the human intestinal flora induce chronic arthritis in rats. Journal of Rheumatology 16, 1061–1068.

Sghir, A., Gramet, G., Suau, A., Rochet, V., Pochart, P., Dore, J., 2000. Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. Applied and Environmental Microbiology 66, 2263–2266.

Shornikova, A.V., Casas, I., Isolauri, E., Mykkänen, H., Vesikari, T., 1997a. *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children. Journal of Pediatric Gastroenterology and Nutrition 24, 399–404.

Shornikova, A.V., Casas, I.A., Mykkänen, H., Vesikari, T., 1997b. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatric Infectious Disease Journal 16, 1103–1107.

Shu, Q., Qu, F., Gill, H.S., 2001. Probiotic treatment using *Bifidobacterium lactis* HN109 reduces weanling diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model. Journal of Pediatric Gastroenterology and Nutrition 23, 171–177.

Siitonen, A., Vapaatalo, H., Salminen, S., Gordin, A., Saxelin, M., Wikberg, R., Kirkkola, A.L., 1990. Effect of *Lactobacillus* GG yogurt in prevention of antibiotic associated diarrhea. Annals of Medicine 22, 57–59.

Simelyte, E., Rimpiläinen, M., Lehtonen, L., Zhang, X., Toivonen, P., 2000. Bacterial cell wall-induced arthritis: chemical composition and tissue distribution of four *Lactobacillus strains*. Infection and Immunity 68, 3535–3540.

Sloan, E., 2000. The top ten functional food trends. Food Technology 54, 33–62.

Suau, A., Bonnet, R., Sutren, M., Godon, J.-J., Gibson, G., Collins, M.A., Dore, J., 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Applied and Environmental Microbiology 65, 4799–4807.

Surawicz, C.M., Elmer, G.W., Speelman, P., McFarland, L., Chinn, J., van Belle, G., 1989. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*. A prospective study. Gastroenterology 84, 981–988.

Tepper, B.J., Trail, A.C., 1998. Taste or health: a study on consumer acceptance of corn chips. Food Quality and Preference 9, 267–272.

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. Alimentary Pharmacology and Therapeutics 13, 1103–1108.

Wheeler, J.G., Bogle, M.L., Shema, S.J., Shirrell, M.A., Stine, K.C., Pittler, A.J., Burks, A.W., Helm, R.M., 1997a. Impact of dietary

yogurt on immune function. American Journal of the Medical Sciences 313, 120–123.

Wheeler, J.G., Shema, S.J., Bogle, M.L., Shirrell, M.A., Burks, A.W., Pittler, A., Helm, R.M., 1997b. Immune and clinical impact of *Lactobacillus acidophilus* on asthma. Annals of Allergy Asthma and Immunology 79, 229–233.

Winkelstein, A., 1956. *Lactobacillus acidophilus* tablets in the therapy of functional intestinal disorders. American Practitioner and Digest of Treatment 7, 1637–1639.

Yeomans, M.R., Jackson, A., Lee, M.D., Steer, B., Tinley, E., Durlach, P., Rogers, P.J., 2000. Acquistion and extinction of flavour preferences conditioned by caffeine in humans. Appetite 35, 131–141.