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Effect of probiotics and functional foods and their use in different diseases

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Abstract

The use of functional foods (probiotics and prebiotics) has been demonstrated to be effective for the treatment or control of several diseases. Further well designed trials to examine the effects of different probiotic components are required. It is important to establish separate functions and to gain further insight into the underlying mechanisms that include competitive exclusion and modification of colonic microflora. For a very long time Russians have used kefir for the treatment of a wide range of illnesses. This paper attempts to review the use of probiotic and functional foods in different diseases, with a special emphasis on kefir. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

In the context of a workshop held in Barcelona in 1995, the International Life Science Institute (ILSI) summarized and concluded that colonic microflora performs a variety of unique activities and that it is more important to evaluate these activities than to analyze bacterial composition in terms of genera, species or strains [1]. The gut is an obvious target for the development and testing of functional foods because it acts as an interface between

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diet and the metabolic pathways of human health. Probiotics are foods that contain live bacteria, which are beneficial to health [2]. According to another definition, a probiotic is a live microbial food supplement that beneficially affects the host animal by improving the microbial balance and they are used in fermented dairy products and cheeses [3].

Gorbach [3] stated the criteria for an ideal Lactobacillus (L.) strain for use in the dairy industry. The characteristics of this kind of microorganism are: resistance to acid and bile, attachment to human epithelial cells, colonization of the human intestine, production of antimicrobial substances, good growth characteristics, beneficial effects on human health.

On the other hand, we have prebiotic substances such as lactulose, lactitol, xylitol, inulin and certain non-digestible oligosaccharides that selectively stimulate the growth of bifidobacteria in the colon. The latter are in the market on the basis of their claimed benefits: alleviation of lactose maldigestion, increased resistance to gut invasion by pathogenic species of bacteria, stimulation of the immune system and possible protection against cancer. Much more work is needed on the mechanism of immunomodulation and of competitive exclusion and microflora modification [2,3]. The other potential functional effects of prebiotics are on the bioavailability of minerals, and on lipid metabolism [4]. Potential health benefits may include reduction of the risk of intestinal infectious diseases, cardiovascular disease, non-insulin-dependant diabetes, obesity, osteoporosis and cancer [5]. Bouhnik et al [6] demonstrated that the administration of Bifidobacterium (B.) sp. substantially increases the proportion of bifidobacteria in the colonic flora, but the concurrent administration of inulin does not enhance this effect. On the other hand, he observed that the addition to the diet of small amounts of indigestible oligosaccharides, which do not induce digestive symptoms, alters the concentration of bifidobacteria and the intracolonic fermentation metabolism, although the benefitial effects on the host have yet to be proved [7].

A large number of bifidobacterial products are available in the market in the form of milk or yogurt. Also, special freeze-dried pharmaceutical dietary preparations are available as tablets. They contain viable cells of Bifidobacterium sp. alone or in combination with other organisms and are used for the therapy of gastrointestinal disorders, side effects of antibiotic or radiation therapy and chronic constipation, among others [8]. The objective of products of functional foods such as probiotics is to control cellular well-being and boost implementing the endogenous defense capacities of cells. Some of the proposed mechanisms of action of functional foods are shown in Fig. 1.

Probiotics and prebiotics are of current interest because they offer alternatives for the management of different gastrointestinal disorders, multiple antibiotic resistance and overwhelming infections in hospitalized patients [9] (Table 1).

2. Probiotics

2.1. Age

It was demonstrated that the fecal bacterial profile in children was different than in adults, with more Bifidobacteria than Bacteroides and higher counts of facultative anaerobes. With the exception of ammonia concentration and beta-galactosidase activity, the biochemical

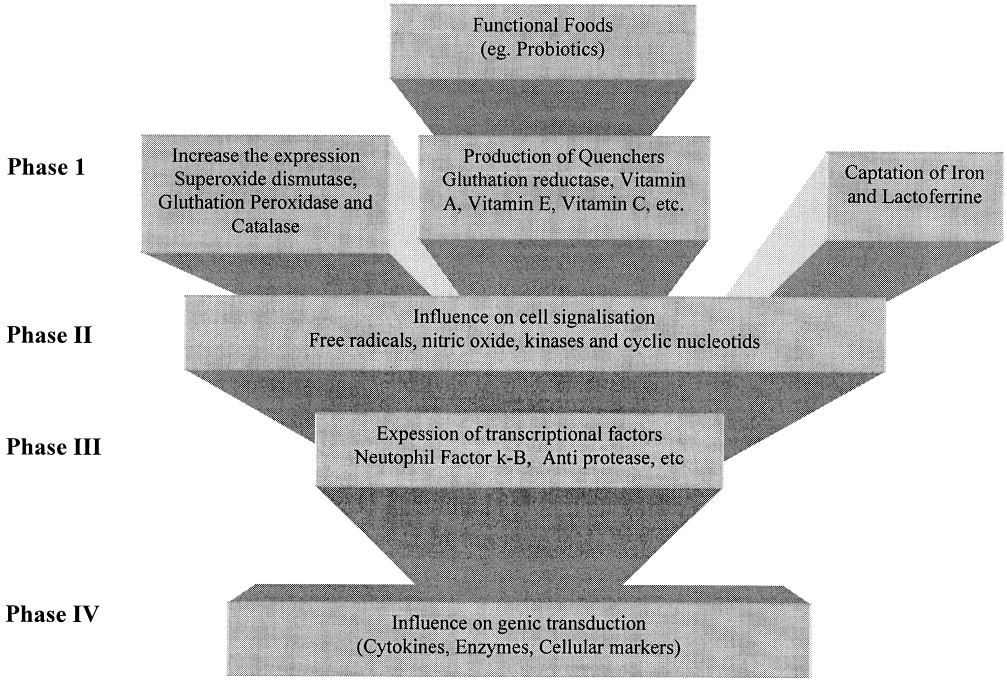

Fig. 1. Putative mechanisms of the action of functional foods. Cells adapt their own intracellular biochemical armature by increasing the global antioxidant armature and thus control in cascade the redox intracellular signalisation by decreasing the oxygen tension, the activation of transcription factors and finally controlling the hyper-activation of genes that controlled the innate immunity.

parameters are comparable to those found in adults. Diseases, like gastroenteritis, are able to change the biochemical and bacterial profile [8,10]. After antibiotic therapy, the frequency of dysbacteriosis in children is very high. The use of probiotic foods can re-establish the equilibrium of colonic microflora and may help to control the adverse effects of antibiotic therapy. With the improvement in health care, living standards, and socioeconomic status, more adults are living to old age. One of the most remarkable changes with aging is the frequent development of atrophic gastritis and the inability to secret gastric acid. This process affects approximately one-third of older adults in the U.S. and only recently was recognized to be due to infection by Helicobacter pylori in the majority of cases. The lack of gastric acid in atrophic gastritis may lead to small intestinal bacterial overgrowth and influences the absorption of a variety of micronutrients. Lactose maldigestion is a frequent condition in older adults [11]. So, the use of probiotics in this population would yield good results.

2.2. Gastrointestinal disorders

2.2.1. Ulcer

The prevalence of peptic ulcer disease has been associated with diet. It was demonstrated that individuals with verified ulcer had a lower intake of fermented milk products and

Table 1
Mechanisms of probiotic functionality and its beneficial effects

Mechanisms of functionality	Beneficial Effects
Antimicrobial activity	• Control of rotavirus and Clostridium difficile • Control of ulcers related to Helicobacter pylori • Antibiotic therapy • Treatment of diarrhea associated with travel
Colonization resistance	• balancing of colonic microbiota
Immune effects	
• Adjuvant effect	• vaccine adjuvant effect
• Cytokine expression	
• Stimulation of phagocytosis by peripheral blood leucocytes	• enhanced immune response
• Secretory IgA	• enhanced immune response
Influence on enzyme activity	• reduction of fecal enzymes implicated in cancer initiation • reduction of serum cholesterol
Enzyme delivery	• amelioration of lactose malabsorption symptoms
Antimutagenic effects	
Antigenotoxic effects	

vegetables and a higher intake of milk, meat and bread than normal subjects. A high intake of fermented milk products was associated with decreased risk of ulcer, whereas an increased risk of ulcer was noted with high milk intake [12]. Other studies demonstrated that L. acidophilus and B. bifidum in concentrations of 10^9 bacteria act as an "ecological" therapy for gastritis and duodenitis [13].

2.2.2. Diarrhea

A fermented product containing L. acidophilus has been shown to inhibit the growth of pathogenic organisms like S. dysenteriae, S. typhosa and E. coli. The beneficial effect of probiotic feeding in bacterial diarrhea may be due to the antimicrobial substances produced by L. acidophillus, which might neutralize the enterotoxins of E. coli [14]. Silva et al. [15] concluded that the protection against S. enteritidis subsp. typhimorium observed in conventional and gnotobiotic mice treated with bifidus milk was not due to the reduction of intestinal populations of the pathogenic bacteria. However, Gallaher et al. [16] studied the characteristics of the bifidobacteria and their stability in dairy preparations and on the potential effects of bifidobacteria and L. acidophilus in the reduction of colonic flatus production in humans and modulation of C. difficile diarrhea.

2.2.3. Food allergy

Some studies demonstrated that probiotic bacteria (L. GG) may promote endogenous barrier mechanisms in patients with atrophic dermatitis and food allergy, and by alleviating intestinal inflammation, may act as an useful tool in the management of food allergy [17]. For the treatment of gastrointestinal disorders, the antigenicity of the diet should be taken into consideration, when introducing novel probiotic functional foods. It has been demonstrated that probiotics (L. GG) not only restore aberrant macromolecular transport, but they also have a specific effect on mucosal degradation depending on the dietary antigen [18].

2.2.4. Helicobacter pylori infection

In cases of H. pylori associated pathology, the deficiency of Lactobacillus sp. in the stomach was found. Studies also revealed a decrease in the population level of Bifidobacteria sp. with the simultaneous increase of opportunistic enterobacteria and changes in local immunity. So, it can be concluded that the correction of microecological and immune disturbances with probiotic preparations containing bifidobacteria and lactobacilli, may be yielded good results [19]. On the other hand, it has been demonstrated that a certain amount of time is necessary for H. pylori to come in contact with the gastric epithelium and that the composition of the gut flora is important for the establishment of H. pylori infection [20]. On the basis of clinical criteria, the use of probiotics containing lactobacillus and bifidobacteria, simultaneously with "triple" antibacterial therapy, has been found to be curative in the treatment of H. pylori associated gastroduodenal pathology in children. The prescription of bifidobacteria containing probiotics is recommended in the early stages of etiotropic therapy [20a]. Studies performed in vitro have demonstrated that L. salivarius but not L. casei nor L. acidophilus proved to be capable of producing high amounts of lactic acid and thus completely inhibiting the growth of H. pylori in a mixed culture. Based on these findings, L. salivarius was found to be a potentially effective probiotic against H. pylori [21]. However, Bazhenov et al [22] found the presence of high antagonistic activity in strains L. casei 925, L. plantarum 8RA-3, L. fermentum BL-96 and L. 90265. These results were confirmed in another study in which it could be observed that L. salivarius inhibited both the attachment and IL-8 release in vitro and that H. pylori could not colonize the stomach of L. salivarius infected gnotobiotic BALB/c mice but colonized in large numbers and caused active gastritis in germ free mice. Moreover, L. salivarius given after H. pylori implantation could eliminate colonization by H. pylori [23]. Other researchers suggested that the growth of H. pylori may be suppressed by the immunological system and eradicated by lactobacilli previously inhabiting the stomach [24]. In another study, Midolo et al. [25] demonstrated that six strains of L. acidophilus and one strain of L. casei subsp. rhamnosus inhibited H. pylori growth, whereas B. bifidus, Pediococus pentosaceus and L. bulgaricus did not. Further more, natural antibiotic lactoferrin, found in bovine milk, was found to be bacteriostatic for H. pylori when cultured at concentrations >0.5 mg/ml. So, it has a significant antimicrobial activity against Helicobacter species in vivo and in vitro [26].

2.2.5. Lactose intolerance

It is postulated that lactobacillus supplementation could enhance lactose fermentation and thus improve symptoms of lactose intolerance. Studies have shown that the consumption of lactobacilli-containing products reduces the activity of fecal bacterial enzymes, including beta-glucuronidase, nitroreductase and azoreductase. Colonic flora may adapt quickly to and metabolize most of the lactose malabsorbed in the small intestine. L. acidophilus result in an improvement of in vitro lactose fermentation when a stable bacterial population and/or an adaptation of colonic flora to lactose load is not established. L. acidophilus strain LA-1 improves lactose digestion during the initial phase, and may suggest that metabolic alteration by lactobacilli supplementation is most likely to occur when colonization resistance is reduced, that is, when a stable microflora has not been established [27].

2.3. Cancer

A high percentage of human tumors is reported to be related to dietary habits. Specific strains of lactic acid bacteria used to ferment milk are promising candidates that may be antimutagenic and anticarcinogenic. Different fermented fresh yogurts containing viable bacteria (L. delbrueckii sp. bulgaricus and S. thermophilus or L. acidophilus and Bifidobacteria) showed protective effects as well. Other fresh fermented milk products (buttermilk, kefir and Dickmilch) were demonstrated not to be antimutagenic. These results imply that some bacteria used in milk processing have an antimutagenic potential and that this property is specific for the bacterial strain [28]. The antimutagenic effects of milk and milk cultured with Bifidobacterium or Lactobacillus strains towards the mutagenicity induced by direct mutagens and dietary indirect mutagens were investigated. The results showed that each cultured milk sample and control milk had a significant antimutagenic effect but uninoculated milk had a greater inhibitory effect than cultured milks towards dietary indirect mutagens [29]. In another study the incidence of aberrant crypts in animals fed different skim milks (skim milk fermented with Bifidobacteria sp. Bio (Danone strain 173010) and skim milk fermented with lactic acid bacteria) were compared to uninoculated skim milk. The tested diets reduced the incidence of aberrant crypts by 49%, 61% and 51% respectively [30]. The mix of Bifidobacteria sp. Bio (Danone strain 173010), casein and calcium probably contribute to the total protective effect of dairy products against induced mutagenicity [31]. However, Gallaher et al. [16] did not find any effect of the bifidobacteria and lactobacilli on protection from development of aberrant crypts in rats. Other bacterial strains (L. helveticus, Bifidobacteria sp. and a mix of S. thermophilus and L. bulgaricus) induced a significant, although variable, reduction in the growth rate of HT-29 cells, which resulted in a 10–50% decrease in the cell number at steady-state [32].

2.4. Immune system

Fukushima et al. [33] found that the intake of bifidobacteria can enhance local production of IgA in milk and the intestine, which may help to protect both pups and dams from exposure to food antigens. A study conducted to determine the effect of the consumption of milk fermented with L. casei strain Shirota on the composition and metabolic activities of the intestinal microflora and immune parameters in humans, demonstrated that the consumption of L. casei Shirota fermented milk was able to modulate the composition and metabolic activity of the intestinal flora and indicated that this milk did not influence the immune system of healthy immunocompetent males [34]. These results are in accordance to the ones obtained by Schiffrin et al. [35] who demonstrated that L. acidophilus La 1 and B. bifidus Bb 12 do not modify lymphocyte subsets in humans, but phagocytosis of E. coli sp. was enhaced in both groups. On the contrary, another study suggested the involvement of lactic acid bacteria in cytokine production under healthy conditions. They found that yogurt intake containing 10^{11} bacteria led to increase in 2′–5′ synthetase activity in human blood mononuclear cells. This result may suggest an interferon action in a peripheral way [36].

3. Kefir as a probiotic

Kefir is the product of fermentation of milk with kefir grains. The composition of kefir grains is variable and not well defined. It is described as a symbiotic association between lactic and acetic bacteria and yeast. To study the distribution of kefir bacteria in kefir grains, immunofluorecence microscopic studies were carried out. The authors found that Kefiran-producing, encapsulated L. kefiranofaciens that are located all over the grain and increased in the center, while L. kefir populated only a small region at the surface layer [37]. On the other hand, Lactobacillus sp. KPB-167B was found to be similar to kefiran in the production of a capsular polysaccharide. Lactobacillus sp. KPB-167B could grow and produce capsular polysaccharide with a better yield than L. kefiranofaciens, which suggested that it is suitable for kefiran production [38]. Fermented milks (yogurt, kefir, sour milk) compared with milk gave higher values of non-protein nitrogen and free amino-nitrogen content after pepsin digestion in vitro. Diets based on casein, milk and fermented milks showed the maximum increment of body mass in rats which had been fed the diets based on fermented milks. The highest values of the protein utilization index were found in the animals kept on fermented milk products. The favorable protein utilization and body mass increment in animals on fermented milk diets were attributed to a better digestibility of proteins in these products [39].

3.1. Cancer

A water-soluble polysaccharide (KGF-C) from kefir grains was shown to have the property of retarding tumor growth in vivo when administered orally. Oral immune enhancement by KGF-C is elucidated probably through T-cell and not through B-cell participation [40]. On the other hand, KGF-C was tested on a delayed-type hypersensitivity (DTH), after an oral administration in mice. KGF-C caused an increase in DTH response in intact mice and also tumor-bearing mice. The growth of tumor inoculated after DTH test was markedly inhibited in the groups with high DTH response. A significant correlation between the DTH response and the antitumor activity was observed in intact mice [41]. The KGF-C inhibited the growth of the solid tumor S-180 by 21–81% and 40–59% in Ehrlich carcinoma, compared with the control mice. The mechanism of antitumor activity was considered to be host-mediated because of the lack of direct in vitro effect on tumor cells [42]. The antitumor activity of kefir was investigated in Caucasus. YK-1 activated the immunosuppressive activity of spleen cells of mouse treated with immunosuppressive substances [43].

3.2. Infants

It is recommended that whole kefir may be used for feeding premature infants because of good tolerance, and adequate weight gain. A high content of indispensable fatty acids in blood serum were attained [44].

3.3. Immune system

The addition of peptides obtained from nervous tissues of squids was evaluated in experiment milk medical and prophylactic products (milk and kefir). The addition of gangliin to the milk and kefir causes stimulation cellular and humoral factors of immunity in laboratory animals [45]. On the other hand, kefir contains an active substance which enhances IFN-beta secretion of a human osteosarcoma line MG-63 treated with chemical inducer poly I: poly C. The active substance in the fermented milk was identified to be sphingomyelin (SpM) [46].

3.4. Gastrointestinal disorders

3.4.1. Lactose intolerance

Feeding kefir with beta-galactosidase activity resulted in a 30% enhancement of the mean post-prandial plasma galactose peak concentration, as well as a 23% greater mean area under the galactose response curve when compared with heat-treated grains. Kefir does not induce intestinal B-galactosidase activity or intestinal lactose-hydrolyzing bacteria by lactose feeding. These results give direct evidence of an enhanced lactose digestion and absorption in native fermented milk products due to the microbial beta-galactosidase activity [47]. Lactose was decreased in all fermented products, while galactose increased from traces in milk up to 1.3 g/100 g in yogurt. Buttermilk, kefir and ropy milk showed 26, 30 and 20% decreases in lactose content. Eight lactose intolerant individuals showed symptoms of abdominal distress and diarrhea following consumption of 500 ml of low fat milk whereas ingestion of the same quantity of yogurt or acidophilus milk did not result in any symptom. Fermented milk products should be considered in formulating diets for lactose intolerant subjects [48].

3.4.2. Intestinal infections

Yogurt inhibited the growth of Salmonella and Shigella very effectively even when the yogurt had been heated to 100°C for 15 minutes, whereas milk and other fermented milk products showed a lower ability to inhibit the growth of pathogens. Yogurt plus human gastric juice greatly depressed the growth rate of the pathogens; after 30 minutes no more colonies were formed. Even kefir or ropy milk plus gastric juice showed inhibition of S. typhimurium after one hour. Yogurt contains some antimicrobial compounds that inhibit the growth of pathogens and this inhibiting property is enhanced by the addition of human gastric juice [49]. Lactococcus lactis DPC3147, a strain isolated from an Irish kefir grain, produces a bacteriocin with a broad spectrum of inhibition. The bacteriocin produced is heat stable, particularly at low pH and inhibits nisin-producing (Nip+) lactococci. Bacteriocin producing starters produced acid at rates similar to those of commercial strains. The level of lacticin 3147 produced in cheese remained constant over 6 months of ripening and correlated with a significant reduction in the levels of nonstarter lactic acid bacteria. Such results suggested that these starters provide a means of controlling developing microflora in ripened fermented products [50]. In patients with chronic enteritis the content of E. coli, bacteroids and bifidobacteria diminished while that of staphylococcus, enterococcus and fungi rose. In this study, patients received different probiotics (shubat, koumiss and kefir). Kefir adminis-

tration did not produce significant shifts in the intestinal microflora. Lactic acid products were ineffective in fungus and proteus dysbacteriosis [51]. However, Aciprole (L. acidophilus + kefir grains) was used to manage antibiotic dysbacteriosis as an adverse reaction of antibacterial therapy. The therapy under the antibiotic + Aciprole regimen lowered the frequency of dysbacteriosis events and their severity. The fact that the use of this probiotic with antibacterial therapy prevented the development of the clinical signs of dysbacteriosis of practical importance [52].

3.4.3. Helicobacter pylori infection

Fresh kefir appear to have a stimulatory effect on the motor and emptying function of the gastric stump whereas milk and milk whey, cottage, cheese and butter was shown to have inhibitory effects on this function. So, the dietetic management of the patients undergoing gastric operations or with H. pylori colonization should be carried out on a strictly individualized basis [53].

4. Conclusions

Probiotics have been studied widely for their use in different pathologies. Kefir, as a fermented product proved to be useful to control several diseases. Even though, there are many studies about probiotics and their properties, the results are still controversial and few studies have been carried out in human beings. On the other hand, the methodology used in most of the studies, namely, the study of fecal samples alone, is apparently not sufficient for elucidation of the gastrointestinal ecology of probiotic bacteria [54]. Even more, fecal persistence may not necessarily reflect in vivo colonization and may not be a prerequisite for all form of immune reactivity [55].

The observation that kefir stimulates gastric emptying, and that the administration of kefir together with other probiotic bacteria has a beneficial effect in the control of certain infections suggest that kefir should help in the treatment of the H. pylori infection.

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