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Review

Diet and bacterial colonization: Role of probiotics and prebiotics

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The intestinal mucosa functions as a defensive barrier that limits dietary antigen transport and microbial pathogens from colonizing enterocytes. The potential role of lactic acid bacteria, specifically Bifidobacteria, *in probiotic and prebiotic functional food supplements is reviewed in the context of bacterial colonization, adherence, and disease prevention. This article reviews the mechanisms of action and optimization of methods that will lead to a new generation of biotic products with enhanced biologic properties and well-being foods.* (J. Nutr. Biochem. 9:668–675, 1998) © *Elsevier Science Inc. 1998*

Keywords: *Bifidobacteria*; *Lactobacilli*; prebiotics; probiotics; intestinal colonization; adhesion

Introduction

Gastrointestinal microflora consist of hundreds of different types of microorganisms and are a biologically important component of the body. At the beginning of this century, Metchnikoff[1] observed that the consumption of fermented milks had beneficial effects associated with the autodigestion of lactose. Through a process of fermentation, the metabolites of these complex microbes have varying consequences on host health. Proteolytic species, for example, produce toxic compounds, whereas metabolism of short-chain fatty acids may result in an increased energy yield.[2] The effects a species of organism have in the microecology of the gut depend to some extent on the organism's ability to survive and preferably multiply in the intestinal tract (*Figure 1*).

Modern perspectives on consumption of fermented milk supplements (e.g., yogurt, fluid milk) are aimed at consumer well-being using products enriched with acid bacteria (*Lactobacilli*), particularly *Bifidobacteria*, that normally inhabit the intestinal tract of human infants and adults. A probiotic, defined as a live microbial food supplement, benefits the host by improving its intestinal microbial balance.[3] In contrast, a prebiotic is a nondigestible nutritional compound [e.g., transgalactosylated and fructooligosaccharides (FOS)] that selectively stimulates the growth of endogenous lactic acid bacteria and *Bifidobacteria* to improve the health of the host.[4] The concept of synbiotics has been proposed recently to characterize colonic foods with prebiotic and probiotic properties as health enhancing functional foods.[5]

Biotechnological advances with these health supplements require more detailed knowledge of the role that enteric feeding and the microenvironment play in host defense than is currently available. Harnessing the biotechnology of biotic supplements introduces new perspectives that require scientific investigation of interactions with genomic, biochemical, cellular, and physiologic functions that promote human health and disease prevention.

Functional effects of probiotics and prebiotics

Research and development of biotic products have been increasingly focused on evidence of functional benefits including resistance to infection, antibacterial activity, and improved immune status. Health claims associated with probiotic supplements include prevention of diarrhea[6,7] and colitis,[8] antitumorigenic effects,[9] and cholesterol reduc-

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J. Nutr. Biochem. 9:668–675, 1998

655 Avenue of the Americas, New York, NY 10010

0955-2863/98/$19.00
PII S0955-2863(98)00058-8

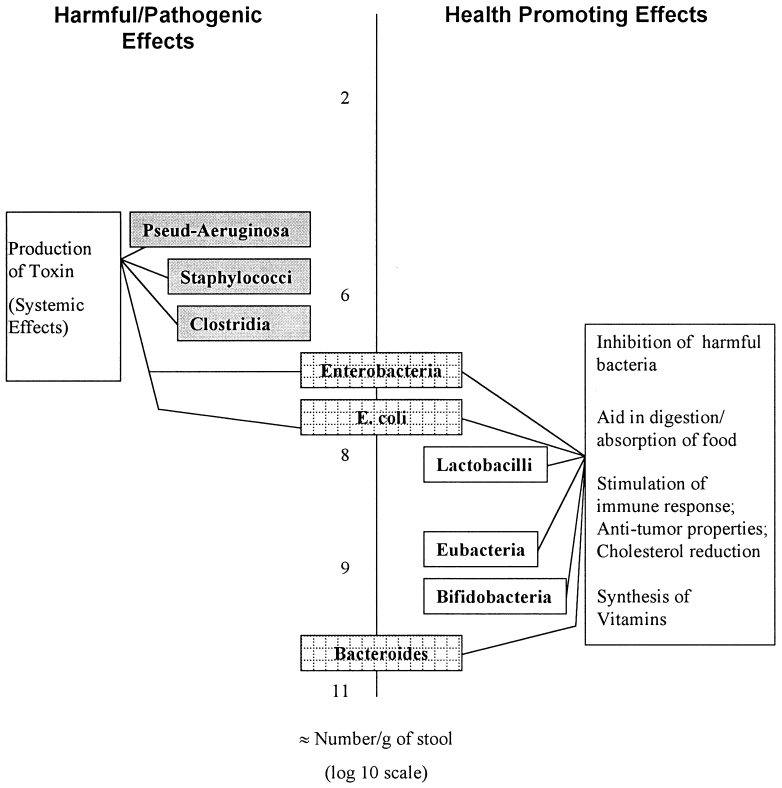

Figure 1 Dichotomy of microflora based on potentially toxic or beneficial properties. Modified from Gibson and Roberfroid (5).

tion.[10] Balanced colonic microflora and immunostimulation are major functional effects attributed to the consumption of probiotics. The concept of prebiotics is relatively new; it developed in response to the notion that nondigestible food ingredients (e.g. nondigestible oligosaccharides) are selectively fermented by one or more bacteria known to have positive effects on gut physiology. Bacteria fed by a preferential food substrate have a proliferative advantage over other bacteria. Prebiotics selectively modify the colonic microbiota and can potentially modulate lipid metabolism.

Gastrointestinal functional effects of prebiotics have been shown almost exclusively for fructooligosaccharides (derived from chicory). Chicory FOS (chiFOS) are prebiotics that have a bifidogenic effect.[11] This class of nondigestible oligosaccharides resists hydrolysis, reaches the colon intact, and as short-chained oligosaccharides is extensively fermented in the colon by resident symbiotic anaerobic bacteria. The fermentability and bifidogenic effect of FOS have been confirmed with in vitro[12,13] and in vivo[14,15] studies. The results have verified increases in short-chain fatty acids, lower pH, and proliferation of *Bifidobacteria* spp in the colon and fecal flora. All of these functional effects are of interest and deserve further study. However, functional food science demands rigorous scientific investigation of biotic products in humans and animals to validate their importance in human health. Evidence from a few rigorously designed prospective cohorts and randomized double-blinded studies tentatively indicate that a few well characterized probiotic strains have documented health-promoting effects.[16] Dose-response studies also have been published.[17]

Perspectives on probiotic applications

Lactic acid bacteria traditionally used in the production of fermented foods constitute roughly 25% of human dietary compounds. The application of probiotic organisms has been extended to uses outside of human nutrition, including animal production and plant protectives in agriculture.[18,19] Current perspectives on biotechnical applications of probiotic products require further in vitro and in vivo investigation to evaluate the safety of using wild-type organisms or those obtained by genetic engineering. Selection of the best adapted and safely performed laboratory strains depend on: (1) selection of strains with demonstrated probiotic effects; (2) evidence of health-promoting effects (e.g., production of essential amino acids, antitumor activity, and vitamins); and (3) strains with food protective activities (growth inhibition of food spoilage or poisoning bacteria).[20] Successful probiotic bacteria are generally identified by additional strain properties including the ability to survive gastric conditions, colonize the intestine, and adhere to intestinal epithelium.[21] The promising use of probiotic supplements and the composition of complex biological preparations may increasingly rely on gene engineering for the treatment dysbacteriosis of various etiologies in the future. Modern genetic techniques will include advanced typing procedures that are based on genetic tagging (16S rRNA, 16S DNA) with oligonucleotides.[22]

Mechanisms of action from fermenting bacteria

Mechanisms by which probiotic and prebiotic supplements affect the microecology of the intestinal tract are not well

studied, but at least four mechanisms of action have been observed: (1) antibacterial agents (bacteriocins) that are produced and secreted by probiotic organisms may have an inhibitory effect on controlling pathogenic microflora; (2) human breast milk may alter bacterial antagonism for essential nutrients and impede overgrowth of aerobes; (3) stimulation of immune responses (e.g., antibody titers, macrophage activity, T cells, and interferon) may suppress potential pathogens; and (4) specific competition for adhesion receptors to gut epithelium may allow lactic acid bacteria and *Bifidobacteria* to occupy the niche normally required by toxin-producing organisms for colonization.[23]

The various fermenting substrates for specific lactic acid bacteria and *Bifidobacteria* differ in their metabolic potential but in general include the following: (1) production of lactic acid and lesser amounts of acetic and formic acids; (2) production of antimicrobial bacteriocins and fatty acids; and (3) reduction in toxin-producing organisms and bacterial translocation.[24] The mechanisms behind specific benefits include: (1) strengthening of the gut mucosal barrier; (2) gut microflora modification; and (3) adherence to intestinal mucosa with an ability to prevent adherence of pathogen proliferation; (4) modification of dietary proteins by the intestinal microflora; (5) modification of bacterial enzyme activity; and (6) influence on gut mucosal permeability.[25]

The intestinal epithelium acts as a natural barrier to the movement of pathogenic bacteria, antigens, and toxic substances from the gut lumen. When either the normal microflora or epithelial cells are disturbed by dietary antigens, pathogens, chemicals, or radiation, defects in the barrier mechanisms can occur. Altered permeability further facilitates the invasion of pathogens and other harmful substances.[26]

Intestinal barrier function

Most foreign antigens are excluded by the intestinal mucosal barrier, which serves as an important organ of host defense. In addition to its barrier function, the intestinal mucosa is sufficient for assimilating antigens via specialized antigen transport mechanisms in the gut epithelium, particularly in Peyers patches.[27] In the healthy host, pericellular leakage of macromolecules is not allowed because intact intercellular tight junctions maintain the macromolecular barrier.[28] Even in normal physiologic conditions, a small percentage of antigens bypass the defense barrier and are absorbed across the epithelial layer via lysosomal processing of the protein.[29]

The integrity of the defense barrier is necessary to prevent uncontrolled antigen transport. Recently, investigators have determined that nearly 90% of dietary antigen handling evokes a tolerogenic hyporesponsiveness in postnatal development of infants and children. However, in preterm infants intestinal permeability can be dramatically increased post-natally.[30] The increased uptake of dietary and other environmental antigens to the immature gut microvillus membrane may lead to an aberrant immune response and sensitization to the immature gut defense barrier. The intestinal microflora affect gut permeability, offering a rationale for the successful use of fermenting anaerobic bacteria to prevent aberrant antigen transport that can traverse the mucosal barrier.

Adhesion and colonization

The adhesion properties of specific bacterial strains to intestinal cells enhance colonization and have become standard procedure for selecting new probiotic strains. Adherence properties are essential for balance of intestinal microflora, intestinal bacterial enzyme activity, and stabilization of intestinal permeability.[31] Adhesion to intestinal cells can be nonspecific, based on biochemical factors, or specific, involving adhesion molecules on the surface of adherent bacteria and receptor molecules on epithelial cells.[32]

Recent investigations with probiotics

Comparative studies (Table 1) of *Bifidobacteria*, specifically *Bifidobacterium bifidum* (ATCC 15696), *Bifidobacterium breve* (ATCC 15700), *Bifidobacterium infantis* (ATCC 15697), and *Bifidobacterium longum* (ATCC 15708),[33,34] have been grown anaerobically at 37°C in MRS (deMan, Rogosa, Sharp) broth with 0.5% cysteine-HC1 and inoculated at 2.5% into different formulas (based on soy, milk, or nonfat milk) followed by incubation at 37°C for 24 hours. In most cases, the logarithmic phase of growth for all species varied for the first 8 to 12 hours postinoculation. Inhibitory properties were generally observed (possibly associated with immune responses to dietary antigen) during the initial phase of growth. Results suggest that growth characteristics of *Bifidobacteria* are species specific and depend on the amount ingested and duration administered.[33–35]

The potential of lactic acid bacteria for the production of safe and wholesome foods has been the subject of several recent reviews.[36] The human intestinal flora strain *Lactobacillus* GG (ATCC 53103) has been shown to promote local antigen specific immune responses, alleviate intestinal inflammation, and perhaps act in the treatment of food allergy.[37] Several studies have shown that the oral administration of *Bifidobacterium* or *Lactobacillus* organisms improved birth weight gain and piglet mortality in calves and piglets fed probiotic supplements compared with control animals.[18,38]

In vitro studies examining the trophic effects of milk fermented with *Lactobacillus* and *Bifidobacterium* strains indicate trophic and proliferation responses of the intestinal epithelial cell line IEC-6.[39] Other recent model systems that use transgenic and germ-free mice have demonstrated that colonizing bacteria on the small bowel villus can send signals for the maturation of its epithelial cells.[40] Increased folate synthesis by cecal and colonic *Bifidobacteria* also has been demonstrated following ingestion of human milk solids in rats. It is speculated that the rat derives a nutritional benefit because it is coprophagic, so it is less likely that significant folate absorption occurs in the colon.[41]

In breast-fed babies *Bifidobacteria*, *Lactobacilli*, and *Staphylococci* are the predominant organisms in the feces, whereas in formula-fed babies coliforms, enterococci, and Bacteroides predominate. The mechanisms responsible for microbiotic differences in breast and formula infants appear

Table 1 Selected studies showing beneficial effects of prebiotics and probiotics

Investigation	Supplement type	Supplement	Study type	Findings
Duffy et al.[33]	Probiotic	*Bifidobacterium bifidum*	Clinical	Lactose digestion was improved in irritable bowel adolescents and adult volunteers
Saavedra et al.[67]	Probiotic	*B. bifidum*	Clinical	Reduced severity of rotavirus infection in children and stimulated rotavirus-specific antibody response
Isolauri et al.[68]	Probiotic	*Lactobacillus casei* strain GG	Clinical	Shortened the diarrheal phase in children with rotavirus infection and kept urease activity constant
Duffy et al.[46]	Probiotic	*B. bifidum*	In vivo	Reduced rotavirus antigenicity; stimulated precursor B-cell antibody
Mao et al.[69]	Probiotic	*Lactobacillus plantarum*	In vivo	Reduced severity of enterocolitis
Schiffrin et al.[70]	Probiotic	*Lactobacillus acidophilus* La1 and *B. bifidum Bb12*	Clinical	No modification in lymphocyte subsets, but enhanced leukocyte phagocytosis of *Escherichia coli* spp.
Kabir et al.[71]	Probiotic	*Lactobacillus salivarius*	In vivo	Inhibited colonization by *Helicobacter pylori* in the stomach
Singh et al.[72]	Probiotic	*B. longum*	In vivo	Oral administration caused antitumor activity in colon
Wang and Gibson[12]	Prebiotic	Oligofructose and inulin	In vitro	Had stimulatory effect on *Bifidobacteria*, while maintaining *E. coli* and *Clostridium* populations at relatively low levels
Gibson and Wang[73]	Prebiotic	Oligofructose	In vitro	Oligofructose, compared with sucrose and inulin, preferentially enriched for *Bifidobacteria*
Ohta et al.[74]	Prebiotic	Fructooligosaccharides	In vivo	Improved recovery from anemia and increased absorption of iron (Fe), calcium, and Magnesium, in Fe-deficient anemic rats
Ozcelik et al.[75]	Prebiotic	Lactulose	In vivo	Oral lactulose treatment prior to surgical trauma reduced bacterial translocation to mesenteric lymph nodes and portal venous blood
Bovee-Oudenhoven and Van der Meer[76]	Prebiotic	Lactulose (and calcium)	In vivo	Combination of dietary lactulose and calcium phosphate were protective against *Salmonella* infection
Campbell et al.[15]	Prebiotic	Oligosaccharide	In vivo	Increased cecal short-chain fatty acid concentration, lowered pH, and increased *Bifidobacteria*

to be related to the acid-based properties of the formula and immunologic proteins including lactoferrin, oligosaccharides, and secretory immunoglobulin A (IgA).[42] Despite extensive modifications of cow's milk in the manufacture of modern infant formula, limited studies using lactoferrin-, iron-, and nucleotide-enriched formulas have failed to substantially alter the flora of formula feeding infants closer to that of breast-fed babies.[43] Various possible explanations for this lack of effect include: (1) inactivation of lactoferrin when added to formula; (2) immunologic responses in the intestine to foreign proteins; and (3) need for multifactor action of immunologic factors with proteins and other nutrients for optimal activity and sustained proliferative growth of anaerobes derived from breast milk in the human intestine.[44]

Recent investigations have paid closer attention to stimulation of bifidogenic effects by altering disruptions of gut microflora in disease prevention. Colonization of *B. breve* in very low birth weight infants (VLBW) was examined in a prospective randomized clinical study of 91 VLBW infants.[45] Early administration of *B. breve* significantly decreased aspirated air volume from the stomach and improved weight gain in infants administered the probiotic, which may have been associated with the stabilization of their intestinal flora and accelerated feeding schedules.

Several investigators[46–48] have demonstrated in in vivo animal models and human studies that rotavirus-specific *Bifidobacterium* strains protect against rotavirus-induced diarrhea by reducing rotavirus antigen and stimulating RV-specific IgA antibody. Nonspecific anti-infective mechanisms of host defense also have been reportedly enhanced by ingestion of specific acid-producing bacterial strains.[49] The paucity of data to convincingly show immunomodulatory properties of lactic acid bacteria and *Bifidobacteria* in humans is related to the difficulty in testing wild strains compared with bioengineered strains in human investigations.

Studies in human volunteers investigating the effects of consumption of fermented milk containing different lactic acid and *Bifidobacteria* strains on lactose digestion have concluded that probiotic milks might reduce breath hydrogen response and symptoms from lactose malabsorption.[50] The prolonged ingestion of *Bifidobacteria* strains with or without inulin on enzymatic activities and fecal concentrations of *Bifidobacteria* administered to healthy volunteers substantially increased the proportion of *Bifidobacteria* in the colonic flora but the concurrent administration of inulin did not enhance a bifidogenic effect.[51]

A high percentage of human tumors are reported to be related to dietary habits. One way to improve nutritional impact is to increase the intake of protective factors that inhibit DNA damage. Specific strains of bacteria used to ferment milk are promising probiotic candidates that may be antimutagenic and anticarcinogenic. Studies to date suggest that different fermented fresh yogurts containing viable bacteria (*Lactobacilli Bifidobacteria*) have shown protective effects. In contrast, yogurt products that have been heat treated were not inhibitory.[52] Other milk products (e.g., buttermilk, kefir) were not antimutagenic, which implies that some bacteria used in milk have an antimutagenic

potential and that this property may be specific for the bacterial strain. To demonstrate the antimutagenic effect of fermented milk in the human intestine, fecal mutagenicity and bacterial consumption were investigated recently in six healthy subjects before and during the administration of milk fermented with *Lactobacillus acidophilus* LA-2.[53] The administration of fermented milk caused a decrease (72%) in fecal mutagenicity. Depression of fecal mutagenicity appeared to be related to the increase in *Lactobacillus* and *Bifidobacteria* spp in the feces of all six subjects.

Microbial food supplements with *Bifidobacteria* and *Lactobacilli* that have been used to change the composition of colonic microbiota have, in general, revealed transient changes, and the implementation of exogenous bacteria have been limited to the period of time the supplement is given. In the future, mechanisms of probiotic action and selective approaches to correcting intestinal microflora will increasingly rely on a new generation of wild strain and genetically engineered biotic products with demonstrated antibiotic-resistant and highly adhesive properties.

Recent investigations with prebiotics

Fermentability of oligofructose and inulin substrates in batch culture experiments exert a preferential stimulatory effect on health promoting *Bifidobacteria* while maintaining populations of potential pathogens (*Escherichia coli*, *Clostridia*) at relatively low levels.[12,15,54] Related culture experiments have shown that human milk oligosaccharides are potent inhibitors of bacterial adhesion to epithelial surfaces.[12,55] Despite these promising findings, batch culture experiments have yielded equivocal results and warrant further examination in in vivo model systems to properly evaluate their bioactivity and health effects following ingestion of prebiotic supplements.[11,13]

The ability of *B. bifidum*, *Lactobacilli*, and *Salmonella* spp to grow in media containing FOS (FOS-50 or FOS pure formulation) were conducted in in vitro experiments. *B. bifidum* and *Lactobacilli* clearly inhibited growth of all salmonella serotypes grown in media containing the pure formulation of FOS as the only carbohydrate source.[56] Lactulose or FOS added to infant test formulas (e.g., soy, infant formula with hydrolyzed casein) have in limited synbiotic experiments been inoculated with *Bifidobacteria* strains and incubated at 37°C for 24 hours. Growth in either formula base was inhibited for selected strains of *Bifidobacteria* with lactulose or FOS past 8 hours of inoculation.[57] These results imply that maximal counts and generation times may require additional protein or other nutrients in the substrate, and prolonged growth may be impeded by interference of immunologic responses to dietary antigen.

Breast milk contains nucleotide salts that are present only in minimal amounts in artificial infant formulas prepared from cow's milk and have been suggested as potential cofactors for growth of *Bifidobacteria.* However, supplementation of formula with nucleotide salts has not supported the addition of nucleotides to stimulate selective growth of *Bifidobacteria* in formula-fed infants closer to that resembling fecal flora of breast-fed infants.[58]

Overgrowth of gram-negative toxin-producing bacteria contributes to the pathogenesis of septic multiorgan failure, which is a combination of ischemia, over-activated inflammatory responses, and bacterial toxin production, conditions that enhance bacterial translocation, and inflammatory organ damage in the premature infant.[59] Lactulose and FOS have been shown in animal models to inhibit cell release of inflammatory mediators.[60] Exogenous administration of *Lactobacilli* reduced bacterial translocation and methotrexate-induced enterocolitis in rats.[61] Bacterial translocation has been associated with increased coliform adherence to cecal epithelium in rats by using biochemical fingerprinting methods.[35] Not all cecal coliforms adhered to the epithelium during catabolic stress, implying that for translocation to occur bacterial properties other than adhesion are needed.[62,63]

Supplementing soluble fiber (FOS, xylooligosaccharide) effectively stimulated growth of *Bifidobacteria* and enhancement of cecal epithelial cell proliferation in mice fed semi-elemental test diets.[64] In related experiments, the possible effects of selective decontamination of the digestive tract (SDD) on mortality in rats showed that enterobacteriaceae and yeast were eradicated from the cecal mucosa by SDD, whereas microflora consisting of mainly anaerobes was well preserved within 3 days in rats following severe thermal injury.[65]

Future research perspectives

In addition to digestion and absorption, the gastrointestinal tract performs important immunologic, metabolic, and barrier functions. Because of the important relationship between enteral feeding and the microenvironment, the loss of mucosal barrier function in this area has received increasing attention over the past several years.

Significant advances have been made in understanding normal defense mechanisms of the gut including barrier and immune functions. Bacterial translocation, mediators of inflammatory responses, and microcirculation play important roles in host defense to critical illness.[66] Future research must focus on specific strategies to enhance gut function, prevent loss of gut integrity, and improve patient outcomes. Functional food science must provide better understanding of how implications for early enteral stimulation can alter metabolic states and provide protection against ischemia reperfusion injury in premature newborns, risk of systemic infection particularly sepsis syndrome, and multiorgan failure in critically ill patients.

The systematic investigation of functional claims including bifidogenic effects for FOS, bulking effects for nondigestible carbohydrates, and protective activity against antioxidants demonstrate promising avenues to improve our knowledge about the role of nutrition in maintaining good health and preventing disease. The various health claims promoting biotic supplements will require additional studies involving large populations and long-term trials. In most cases, elucidation of the mechanisms of interaction between functional food components and specific biological functions will make it easier to accumulate convincing evidence to promote functional food science. Because of its direct contact with enteric foods and other environmental antigens, the microenvironment of the gastrointestinal system is a potential target for many experiments in recent years.

Table 2 Important studies for the safety assessment of probiotic and prebiotic functional foods

Type of property studied	Studies	Safety factor to be assessed
Metabolic products	Campbell et al.[15]	Concentrations, safety and other effects
Toxicity	Pool-Zobel et al.[38]	Acute and sub-acute effects of ingestion of large amounts of tested bacteria
Mucosal effects	Beachey[21]; Duffy et al.[46]	Adhesion, invasion potential, intestinal mucus degradation, infectivity in immunocompromised patients
Dose response	Salminen et al.[29]	Dose-response studies by oral administration in volunteers
Clinical studies	Wolf et al.[17]	Potential for side effects, careful evaluation in healthy volunteers, and disease specific studies
Epidemiologic studies	Yolken et al.[7]	Surveillance of large populations following introduction of new strains and products

Improvement of glucose absorption (glycemia and insulinemia), modulation of gastrointestinal transit time, fecal bulking, acidification of colonic content, and control of cholesterol are all recognized potential benefits of enriched functional foods[11] (*Table 2*).

Successful probiotic bacteria used to treat disturbed intestinal microflora and increased gut permeability have undergone limited investigations in children with acute rotavirus diarrhea, subjects with food allergy, and subjects with colonic disorders including colon cancer. Such probiotic microorganisms appear to be promising candidates for the treatment of clinical conditions with abnormal gut microflora and altered gut mucosal barrier functions.[29]

Biotic products may exert their beneficial effects by suppressing harmful microorganisms or stimulating organisms that contribute in a positive way to the nutrition of the host. Both types of mechanistic action deserve further investigation.[13] In the limited experiments to date with synbiotic products (*Bifidobacteria* and FOS; Skim-FO, Bifido-FOS), *Bifido-FOS* has shown promising results in decreasing the number of aberrant crypts, but there has been no clear relationship of aberrant crypts to the numbers of *Bifidobacteria* or toxin-producing organisms.[14]

The concept of eco-immune nutrition also is emerging in cases where modern technology or tube feeding has made early enteral nutrition following postoperative and posttraumatic injury possible.[70] The fermenting flora (anaerobes) are deranged due to disease or antibiotic treatment, and replenishment of beneficial flora is critical in biotic products combining nondigestible carbohydrates, fiber, and amino acids such as arginine and glutamine, where mucosal adhering probiotics are promising avenues of investigation. The presence in milk formula of predigested and desialylated proteins suggests other physiologic intestinal mechanisms of action that open wide possibilities for future development.

The positive value of multifactor action related to the use of combined properties of these supplements may lead to the creation of well-being foods, new forms of drugs, and oral vaccines. Genetic engineering will be increasingly used to produce a new generation of biotic products to promote human health and prevent disease.

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