JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Recent Advances in the Role of Probiotics in Human Inflammation and Gut Health

Prithy Rupa and Yoshinori Mine*

Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT: The gastrointestinal (GI) tract provides residence to an astounding number of bacterial species, which have profound effects on host biology, function, physiology, and immune response. Discovery of "symbiosis factors" from symbionts that facilitate the peaceful coexistence of microbiota and the host immune system are of interest. Symbionts synthesize immunomodulatory molecules that guide maturation of the immune system and have pivotal roles in many biological processes; however, individuals differ in the makeup of their GI microbiota, which is influenced by many external and internal factors such as diet, antibiotic use, and host genetics, which in turn influences health and disease outcomes. Various endogenous, genetic, and environmental factors influence GI development including species composition and health status of neonates, resulting in interactions that occur between the bacteria and the host. Mechanisms of probiotics involved in homeostasis of a balanced immune system have been inconclusive. The probable mechanism of action may be postulated as direct competition between pathogenic bacteria in the gut and/or immune modulation. This review focuses on probiotics in health and disease prevention, especially the biological importance of intestinal regulation of inflammatory processes that may be beneficial in a multitude of disorders both inside and outside the GI tract.

KEYWORDS: *probiotics, micobiota, gut health, inflammation, disease prevention,*

INTRODUCTION

The gastrointestinal (GI) microbiota is a complex collection of microorganisms such as bacteria, fungi, protozoa, and viruses. It is estimated that the intestine of mammals contains approximately 10^{13}–10^{14} microorganisms, which is almost 10 times the number of the actual cells composing the host body. Recognition of the importance of intestinal microflora, especially the health-enhancing "good bacteria"/probiotics, has generated much interest recently in the use of probiotics to promote and maintain health. The microbiome in the GI system is highly variable from person to person.[1−3] Also, the GI tract of animals is populated by an array of microbial communities, which belong to more than 500 different species, and each individual harbors at least 160 different species, which are largely shared among individuals.[4] This mutually interacting system comprising the host cells and the resident microbes (symbionts) are known to play a crucial role in host health. Symbionts that are beneficial to the host organism and those that are involved in promoting health benefits are classified as probiotics. Although the importance of probiotics in host health is now widely recognized and well-known, an understanding of the mechanisms involved is lacking, and little is understood of the molecular host−microbiota interactions that influence host metabolic pathways.

Sophisticated analysis of microbiota associated with human inflammatory diseases is currently a widely discussed topic of interest, and there is mounting evidence that the microbiota is altered in people with chronic inflammatory conditions resulting in chronic diseases such as allergies, asthma, irritable bowel syndrome, diarrhea, gastritis, and inflammatory bowel disease (IBD). Several studies also support a potential role of symbionts in the prevention of intestinal inflammation. Several vital host functions are provided by symbionts, including the digestion of complex polysaccharides, synthesis of vitamins, maintenance of the intestinal epithelial barrier, and resistance to pathogen colonization. Probiotic species that have the most potential applications include *Lactobacillus* and *Bifidobacterium*. Other species with potential applications are *Streptococcus thermophilus*, nonpathogenic strains of *Escherichia coli*, *Enterococcus*, and *Bacillus*, and yeasts such as *Saccharomyces boulardii*. In the gut, a mutual symbiotic host−microbe interaction has coevolved as the bacteria make essential contributions to the host metabolism and symbionts in turn benefit from the nutrient-rich environment in the intestine. Complex interactions are involved between the symbionts and the host.[5] These interactions begin at birth when the sterile epithelium of the GI tract first encounters the symbionts. Both genetic determinants and diet are known to play a crucial role in the interaction. Also, several criteria for classifying a bacteria as a symbiont include that they should be of human origin, nonpathogenic, resistant to various processing conditions, resistant to gastric acid and enzymes, and able to attach to gut epithelial tissue, colonize the GI tract, produce antimicrobial substances, modulate immune responses, and influence metabolic activities of the host.[6,7] Symbionts also decrease luminal pH and secrete bactericidal proteins (bacteriocins).

Symbionts, the GI epithelium, and the host's innate defense responses are all critical for the interplay that governs the host response, and each plays a paramount role in the determination of disease outcomes.[8] Phylogenetically, there are two types of bacterial divisions that are observed in the gut: the Fimicutes

Received: May 2, 2012
Revised: August 8, 2012
Accepted: August 9, 2012
Published: August 16, 2012

dx.doi.org/10.1021/jf301903t | J. Agric. Food Chem. 2012, 60, 8249−8256

and the Bacteriodetes.[9] Within these two communities thousands of phylotypic divisions occur, and bacterial species within these divisions contribute to various host−GI interactions. In particular, the probiotic (health enhancing) potential of the two main intestinal species, lactobacilli and bifidobacteria, have received attention in managing and maintaining human health.[9] The link between these two species and the diversity and functionality of these probiotics in human health has been widely studied.

MODE/MECHANISM OF ACTION OF PROBIOTICS

A clear understanding of how the GI microbiota are assembled and maintained in humans is increasingly relevant to the treatment of complex chronic and inflammatory diseases. It is evident that significant differences exist between the various probiotic bacterial species and strains with regard to their functions. Several mechanisms by which probiotics exert their beneficial effects have been identified in recent years, and selected strains have been screened to treat specific disease conditions; these vary and differ significantly among species. In general, there is a wide range of immunomodulatory, anti-inflammatory, and antimicrobial properties of symbionts that have been observed in vitro and are presumed to exert beneficial health effects. Various mechanisms that contribute to altered immune functions and balanced homeostasis in vivo induced by symbionts may include improved barrier function, modulation of the microbiota itself, and direct effects of bacteria on different epithelial and immune cell types.[10] These effects are discussed with an emphasis on those organisms that have been used to treat human IBD in controlled clinical trials.[11] Three major mechanisms involved with IBD therapy have have been described: (1) Symbionts may block pathogenic bacterial effects by producing bactericidal substances and toxins that adhere to the intestinal epithelium. (2) Symbionts may regulate immune responses by enhancing the innate immunity and modulating pathogen induced inflammation via Toll-like receptor-regulated signaling pathways. (3) Symbionts may regulate intestinal epithelial homeostasis by barrier function and stimulate protective response. Overall, probiotic strains prevent damage and repair and restore the mucosal integrity, increase the epithelial resistance against pathogens, and induce cell proliferation, thereby influencing disease phenotypes and rendering beneficial health effects.

FACTORS INFLUENCING SYMBIONTS

Consumption of probiotic bacteria is the most effective way to re-establish the homeostasis of the GI microflora balance. The ratio between the beneficial microbes (probiotics/symbionts) and harmful microbes would have an important effect on host health. It is of interest to study the various factors that affect the growth and survival of probiotic bacteria that are found to be strain dependent.[12] Earlier research states that symbionts should be no less than 10^6 cfu/mL of bacteria at the time of consumption to exert beneficial effects on host health.[13] Different bacterial strains are proven to be effective for different health conditions and functions. Thus, strain specificity is of prime importance in selecting a probiotic strain. Also, viability is a major criteria to exert beneficial health effects. Other factors that may have an influence on the beneficial effects of symbionts are that the organism must be native to the human GI tract, have the ability to ferment prebiotics, resist acidic (gastric pH 1−4) and alkaline conditions (bile salts present in the small bowel), and survive passage through the stomach in adequate numbers, and also be capable of colonizing at the site of action. To survive, the strain must also be resistant to the enzymes present in the intestine (such as lysozyme) and to the toxic metabolites produced during the process of digestion.[14,15]

ROLE OF PROBIOTICS IN HUMAN INTESTINAL HEALTH

The symbionts play an important role in the health status of humans, especially in intestinal health due to their involvement in various nutritional, immunological, and physiological functions. There are at least about 500 various species of microflora that are part of the normal intestinal flora in the gut. There are 9 times as many bacteria in the GI tract as there are cells in the human body. The species and the number of GI bacteria play important roles in determining health and disease outcomes. A tight association between altered gut microbiota and certain intestinal diseases in human and experimental animal models is observed. Host−symbiont interaction is a two-way dialogue, and in models of colitis, obesity, type 1 diabetes, and other intestinal inflammatory diseases, transmission of an altered intestinal gut microbiota to a genetically intact host is sufficient to modulate the disease outcome.[16,17]

Human GI Tract and Probiotics in GI Health and Disease. The microorganisms colonize every surface of the human body that is exposed to the external environment, including organs such as the skin, the oral cavity, and respiratory, urogenital, and GI tracts. Of these body sites, the GI tract is by far the most densely colonized organ. The jejunal, ileal, and colonic mucosal-associated bacteria differ from fecal bacteria.[18] The fecal samples do not necessarily reflect the intestinal bacterial content of the GI tract. Knowledge of the composition of mucosal-associated bacteria is particularly limited despite its critical role in health and disease by means of its close proximity with the animal host.[19]

Metabolic Pathways for Probiotics in the Gut. The intestinal microbiota interacts extensively with the host through metabolic exchange and co-metabolic activities of the substrates. Major functions of the GI microbiota include various metabolic activities that result in salvaging of energy and absorbing nutrients, effects on the intestinal epithelium, and protection of the host against invasion by harmful microbes. Individual responses to various drug treatments can be strongly influenced by gut microbiome composition, because the microbiome not only provides complementary metabolic pathways for drugs but also acts as a source of pharmacologically active secondary metabolites that can activate the mammalian liver enzymes. Thus, symbionts can influence the toxicity and metabolism of drugs in man. The importance of GI microbiota to host metabolism may be best illustrated by the fact that genetically homogeneous similar animals can have diverse metabolic phenotypes when they have structurally different types of gut microbiota.[20] Various species of the symbionts influence host metabolism, displaying different and varied metabolic interactions, and in turn have an effect on the overall health. Some exert important metabolic activities by extracting energy from otherwise indigestible dietary polysaccharides such as resistant starch and dietary fibers. These metabolic activities also lead to the production of important nutrients, such as short-chain fatty acids (SCFA), vitamins (e.g., vitamin K, vitamin B12, and folic acid) and amino acids, which humans are unable to produce on their own.[21,22] Probiotics

enhance SCFA production, and an increase in SCFA production lowers the gastrointestinal pH, thus improving pathogen resistance and stimulating epithelial cells. The primary function of SCFA is that they are readily adsorbed and play a crucial role in colon physiology and metabolism. Several members of the intestinal microbiota can produce vitamins and provide them to the host, mainly vitamin K and also vitamin B.[23,24] Acidification of the intestinal environment also inhibits the development and colonization of pathogens, as well as the production of toxic elements derived from the metabolic process (ammonia, phenolic compounds, amines, etc.). SCFA provide one of the clearest examples of how nutrient processing by the microbiota and host diet combine to shape the overall immune response. The concentration of intestinal SCFA can be modified by the amount of fiber in the diet, and this in turn affects the composition of the microbiota.[25] Immune cell-associated molecules serve to merge information with regard to the local nutrient/metabolite environment and coordinate local immune responses. Therefore, metabolites are known to play an important role in the immune decision-making process. Furthermore, recently initiated studies are being undertaken to identify key molecular players that link the gut microbiota, metabolism, and host health. Also, gut commensals can regulate host inflammatory responses via SCFA, which are produced by intestinal microbiota-mediated fermentation of dietary fiber.[26]

Diarrhea, Inflammatory Bowel Disease, Irritable Bowel Syndrome, and Gastritis. Basic research on symbionts has suggested several modes of action of the microbiota beneficial for the human body, and clinical research has proven its preventive and curative features in various intestinal-related diseases. Probiotics demonstrate health benefits in humans, and the mechanisms of action in the GI tract include the inhibition of pathogen growth by competition for nutritional sources and adhesion sites, secretion of antimicrobial substances, and toxin inactivation.[27] Consequently, the primary clinical interest in the application of probiotics has been in the prevention and treatment of GI infections and antibiotic-associated diarrheal diseases. Extensive research supports the beneficial role for probiotics in the prevention and treatment of a variety of diarrheal illnesses, such as acute diarrhea (rotavirus induced), antibiotic-associated diarrhea, and travelers' diarrhea. Probiotics such as lactic acid bacteria (LAB) have been used in the treatment of various GI disorders, such as gastric ulcers or antibiotic-associated diarrhea.[28] Strain-specific effects of probiotics have been shown in diarrheal diseases, IBD, irritable bowel syndrome, and *Helicobacter pylori*-induced gastritis.[29] It is also postulated that probiotics release inhibitory substances that inhibit the growth of pathogenic bacteria responsible for diarrhea. As the majority of probiotics naturally inhabit the human intestinal microflora, their use has been regarded as very safe. A number of probiotic strains, such as *Lactobacillus* GG, *Lactobacillus reuteri*, *Lactobacillus casei*, *Saccharomyces boulardii*, and *Bifidobacterium*, have been demonstrated by controlled clinical trials to decrease the severity and duration of acute diarrhea.[30,31] Child-care infants fed a formula supplemented with *Lactobacillus reuteri* or *Bifidobacterium lactis* had fewer and shorter episodes of diarrhea, with no effect on respiratory illnesses. These effects were more prominent with *Lactobacillus reuteri*, which was also the only supplement to improve additional morbidity parameters.[32] Alteration of normal intestinal microflora plays an important role in the pathogenesis of IBD. There is also no known cure; hence, treatment is mainly focused on probiotics as a therapeutic modality. Therefore, modifying the gut microflora with administration of probiotics may treat this condition successfully. Several research groups have reported beneficial effects of symbionts with regard to IBD.[33,34] Also, the symbionts in patients with IBD have been shown to be less stable compared to those of healthy subjects.[35] In the context of IBD, anti-inflammatory bacteria may signal the GI epithelium and perhaps mucosal regulatory T cells or dendritic cells and thereby play a role in immune modulation.[36]

Immune System Modulation. Several studies (in both animal and human subjects) have provided evidence that specific strains of probiotics are able to stimulate as well as modulate several aspects of natural and acquired immune responses. Also, different probiotic strains vary in their ability to modulate the immune system.[37] Diet and associated changes in the gut microbiota are driving increasing incidences of inflammatory immune disease in developed countries.[38] Studies done so far state that probiotic supplementation is able to provide protection from various chronic diseases by down-regulating immune-related inflammatory cytokines or inducing regulatory mechanisms in a strain-specific manner.[39] The intestinal microbiota is the largest source of microbial stimulation in the GIT that exerts beneficial effects on human health. Gut-associated lymphoid tissue (GALT), such as the Peyers patches and small intestinal lymphoid tissue (SILT) in the small intestine, and lymphoid aggregates in the large intestine account for diffusely spread immune cells in the lamina propia of the GI tract and are involved in immune modulation. Contact of these immune cells via mesenteric lymph nodes and gut microbiota overlying the mucosa along with the intestinal epithelium play a major role in the generation of mucosal immune response. Gut microbiota act as primary agents that participate in the development of the postnatal immune system as well as in the development of oral tolerance and immunity. Profiles of cytokines that are secreted by lymphocytes, enterocytes, or dendritic cells that come in contact with various strains and that play a role in immune modulation have been established.[40] Distinct cytokine patterns lead to Th1 and Th2 differentiation of $CD4^+$ helper T cell clones. Inappropriate Th1 and Th2 cytokine responses result in distinct forms of human disease. An intimate relationship exists in the GI tract between gut microbiota signals and Th1 and Th2 cell development and regulation and pro-inflammatory Th17 cells and regulatory $CD4^+$ cells. Balance of pro-inflammatory or regulatory immune responses, Th1/Th2 cells, and gut microbiota in the intestinal mucosa all play important roles in maintaining homeostasis in the gut.

Other Health Effects of Probiotics/Anti-inflammation. A number of beneficial effects have been attributed to the consumption of probiotic products. These include alleviation of allergies and lactose intolerance, protection from GI infections, antimicrobial activity, suppression of cancer, reduction in plasma cholesterol concentrations and, consequently, in coronary heart disease, and improved digestion and nutritional value of foods. *Lactobacillus reuteri* ATCC 55730 is a probiotic lactic acid bacterium that is widely used as a dietary supplement to improve GI, immune, and oral health. Dietary supplementation with the probiotic *L. reuteri* ATCC 55730 induces significant colonization of the stomach, duodenum, and ileum of healthy humans, and this is associated with significant alterations of the immune response in the GI mucosa.[41,42]

Other key benefits of symbionts include protection from infection by producing antimicrobial compounds that kill pathogens in the intestines, vagina, and mouth, reducing the risk of intestinal infections, as well as gum disease and cavities. In addition, symbionts secrete enzymes and acids such as lactic acid and acetic acid, which make the environment in the intestines and vagina unfavorable for infection-causing organisms. Evidence also suggests that probiotics lower the risk of colon cancer, in part due to their role in promoting regular bowel movements and thereby reducing intestinal inflammation.

Allergies and Asthma. In addition to various environmental factors, the intestinal microbiota may be a contributor to allergic disease due to its substantial effect on mucosal immune response. Earlier papers suggest that the makeup of intestinal microflora can be different in individuals with allergic disorders and in those who reside in industrialized countries where the prevalence of allergy is higher.[43]

The "hygiene hypothesis" suggests that the exposure of infants to microbes in the first six months of life helps the immune system mature to be more tolerant to exposure to allergens later in life. Lack of this early neonatal stimulation is postulated to lead to allergies at a later date. The consumption/supplementation of certain probiotics had positive effects on gut barrier function and immune response, thereby exerting beneficial effects on alleviating allergy.[44,45] Also, variation in microbiota composition is seen among individuals that express clinical signs of allergy, which supports the hygiene hypothesis.[46] Two strains (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*) have been validated so far, for alleviating expression of clinical allergic signs in infants.[47] However, these effects are strain specific and thus need to be validated for each strain.[48] We have recently reported the prophylactic effects of heat-killed *E. coli* given intramuscularly or of oral *Lactococcus lactis* on experimental ovomucoid-induced allergy in piglets in the context of altered immune response bias favoring reduced type-2 allergic phenotypes.[49,50] These studies confirm the relevance of testing hygiene and immune response-related hypotheses in pigs in their own right and as large outbred animals similar to humans, which further validates the pig model.

Cancer. Many investigators have evaluated the therapeutic effects of symbionts against diseases such as cancer, infection, and GI disorders. Research suggests that the consumption of probiotic cultures may decrease cancer risk. It has been hypothesized that probiotic bacteria might decrease the exposure to carcinogens by various mechanisms such as altering the environment of the intestine and thereby decreasing the population or metabolic activities of bacteria that may generate carcinogenic compounds; detoxifying ingested carcinogens; and producing metabolic products and compounds that inhibit the growth of tumor cells and stimulate the innate immune system to better defend against cancer cell proliferation. Probiotics may also suppress the growth of bacteria that convert pro-carcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine, reducing the enzymes β-glucuronidase and β-glucosidase and deconjugation of bile acids, or merely enhancing the immune system of the host.[51,52] The ability of probiotics to scavenge free radicals is proven to reduce the occurrence of a number of cancers such as stomach cancer, colon cancer, and mouth cancer. Studies on the effects of symbionts on cancer treatment appear promising, because recent in vitro and in vivo studies have indicated that probiotic bacteria might reduce the risk, incidence, and number of tumors of the colon, liver, and bladder.[53] Also, a recent study demonstrated the in vivo chemopreventive efficacy and immune-stimulating mechanisms of dietary probiotics against DMH-induced colonic tumorigenesis in mice.[54]

Diabetes and Obesity. The connection between gut microbiota and inflammation and homeostasis and its role in the pathogenesis of obesity and diabetes-related disorders is increasingly recognized, and the relationship between diet, gut microbiota, and homeostasis was investigated in models of diet-induced obesity.[55,56] Because obesity, diabetes, and inflammation are related, it can be postulated that the probiotic control of inflammation plays a role in obesity and diabetes prevention. Recent research reported that women who take probiotics during pregnancy have significantly lower odds of developing gestational diabetes, thereby giving them and their offspring a lower risk of developing type-2 diabetes and also giving their children a lower risk of childhood obesity.[57]

Oral Health and Urinary Tract Infections. Probiotics have many positive influences in creating better oral health. Probiotics produce chemicals to inhibit harmful oral bacteria that damage oral hygiene.[58] According to that study different symbionts are needed for therapy in oral mucosal diseases as there is a difference in the microbial attachment sites on the keratinized and nonkeratinized epithelium. Also, probiotics help in binding oral microorganisms to proteins and biofilm formation. They fight plaque formation and its complex ecosystem by compromising and intervening with bacterial attachments. They are a useful tool in the treatment of inflammation and clinical symptoms of periodontitis, especially in high-risk subjects.[59] LAB have been used to treat or prevent infections of the intestinal and genital tracts with different degrees of success.[60] The use of probiotic lactobacilli vaginally and orally has shown great promise in helping to restore and maintain a healthy vagina, and studies have shown that certain strains have the capacity to interfere with the inflammatory pathways as well.[61]

HOST FUNCTION REGULATION

All functions and beneficial effects exerted by the symbionts are related to the host in one way or another. Recent work highlights a direct beneficial effect of gut microbial communities on the host. The microbiome performs a variety of rolesin guarding host health such as maintaining intestinal homeostasis; they act as a defending barrier against invading pathogens, aid in various functions such as digestion and energy harvest from the diet, provide nutritional support for enterocytes,and stimulate the development of the immune system.[62,63] The GIT acts as a barrier against microbial invaders,and the resident microbiota and invading bacteria both interact intimately with the gut epithelium and influence the host cellular and immune systems.[64] Probiotics also increase enzyme production, enhance digestion and nutrient uptake, maintain the host microbial balance in the GI tract through producing various bactericidal substances that compete with the pathogens and toxins for adherence to the intestinal epithelium, promote intestinal epithelial cell survival and barrier function, thereby providing a protective response, and regulate immune response by enhancing the innate immunity. The prime role of probiotics in host-function regulation is the regulation of antigen trafficking and intestinal mucosal microbiota interaction.[65]

Table 1. Strain-Specific Probiotic Organisms Validated in the Treatment of Diseases

clinical conditions	strains of therapeutic use	functional role and mechanism	refs
immune modulation	*L. acidophilus*, *L. casei* F19, *L. casei* Shirota, *L. rhamnosus* LB 21, *B. lactis* HN019, *E. coli* Nissile 1917, *L. plantarum*, *L. delbrueckii*, *L. johnsonii*, *B. bifidum*	↑ phagocytic activity of blood mononuclear and polymorphonuclear cells ↑ production of interferon (IFN) γ cytokine	37−42
traveler's diarrhea	*L. acidophilus*, *L. rhamnosus* LB 21, *L. casei* F19, *L. bulgaricus*, *S. boulardii*, *B. longum*, *E. faecium*	anti-infectious action in the intestine; modulation of innate and adaptive immunity	28−32
allergy	*L.rhamnosus* LGG, *L. acidophilus*, *L. casei* Shirota strain, *B. lactis*, *B. longum* BBS36, *L. lactis*	↑ specific antibody responses following vaccination	43−50
IBD	multispecies (VSL#3), *L. rhamnosus* LGG, *L. salivarius* UCC118	↓ pro-inflammatory cytokines, IFN-γ, TNF-α, and IL-12, and anti-inflammatory effects	33−36, 62
C. difficile colitis	*L. rhamnosus*, *S. boulardii*	stimulates Toll-like receptors; alteration of intestinal flora ↑ antimicrobial activity ↑ intestinal barrier protection	72
anticancer	*L. acidophilus*, *L. casei*, *L. plantarum*, *L. delbrueckii*, *L. gasseri*, *B. longum*, *B. bifidum*, *B. adolescentis*, *B. infantis*	alteration of local metabolic products that affects cell proliferation and apoptosis; regulation of harmful enzyme activity to exert a protective effect	51−54
vaginal health	*L. rhamnosus* GR1, *L. reuteri* RC 14	↑ antimicrobial factors maintenance of a vaginal pH of <4.5	9, 61, 68
intestinal homeostasis	*L. acidophilus*, *L. casei*, *B. bifidum*, *L. plantarum*	↑ in short-chain fatty acids and lactate; activation of signaling pathways	16, 17, 63, 64

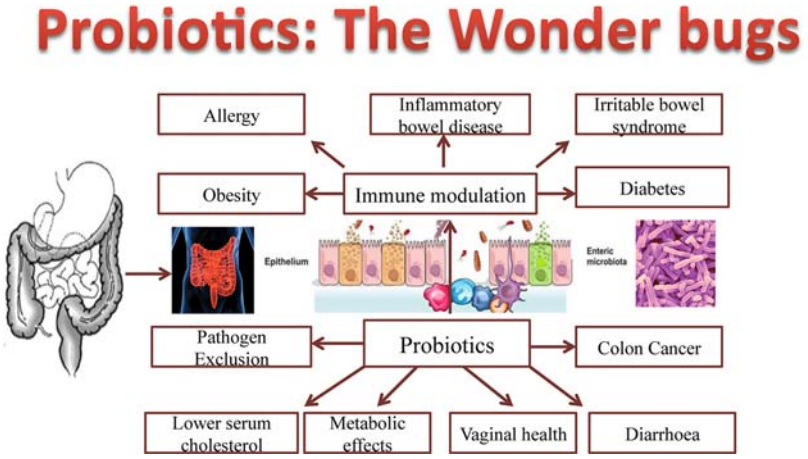

Figure 1. Overall summary of recent advances and role of probiotics in health and disease.

TYPE OF PROBIOTIC STRAIN (PRODUCT CONSIDERATIONS) AND DOSE: DO THEY MATTER? STABILITY ISSUES/BENEFICIAL MICROBIOTA

Beneficial effects with regard to strain specificity of probiotics so far is mostly based on in vitro and animal data where strain differences are evident. Most basic and common attributes such as acid tolerance, sensitivity to therapeutic antibiotics, bile resistance, lactase activity, hydrogen peroxide production, genetic accessibility, production of antimicrobial compounds, and stability in product have all been tested for a variety of strains in vitro.[66] Overall strain specificity does matter, and not all strains have been tested individually or compared for efficacy of each probiotic in a given health condition. Also with regard to dose, various recommendations have been put forward. For *L. reuteri* SD2112 and *Bifidobacterium infantis* 35264 doses of 1 $\times 10^8$ (100 million)/day have been documented as adequate for different health targets. However, product VSL#3 (a probiotic formulation containing multiple probiotic species) is recommended at 1.8×10^{12} (1.8 trillion)/day for management of recurrence of certain IBD conditions. Each study varies, however; a standard dose of live bacteria between 1×10^9 and 1×10^{10} cfu/mL has been widely accepted in most cases.[67] The dosage and duration of therapy must also be considered optimally to enhance and not suppress immunity. Other criteria for probiotics include that they should be isolated from the same species as that of the intended host, and thereby have a demonstrable beneficial effect on the host, they should be nonpathogenic, they should be able to survive transit through the GI tract, and upon storage, a large number of viable bacteria must be stable and able to survive for prolonged periods.

dx.doi.org/10.1021/jf301903t | J. Agric. Food Chem. 2012, 60, 8249−8256

EXTRA-INTESTINAL APPLICATIONS AND OTHER POTENTIAL APPLICATIONS OF PROBIOTICS

Interactions between gut microbes and the host are the subject of intensive ongoing research as these changes influence a variety of diseases. However, probiotics have also been shown to exert beneficial extra-intestinal effects and used to treat various disease conditions. As discussed earlier, symbionts have been beneficial and effective for urogenital health, namely, to prevent, and in some cases treat, urinary tract infection and bacterial vaginosis.[68] Probiotic bacteria have also been known to ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in the levels of blood lipids by inhibiting cholesterol synthesis and redistributing cholesterol from plasma to the liver, thereby lowering blood cholesterol.[69,70] The use of VSL#3 has shown that it is possible to influence urinary oxalate excretion and potentially reduce urinary supersaturation levels and the formation of kidney stones.[71] Studies have also suggested that probiotics can alleviate the signs and symptoms of *Clostridium difficile* infection.[72] Many papers advocate the use of probiotics in the prevention and treatment of *C. difficile* infection along with the standard therapy based on other anecdotal evidence. The use of lactic acid bacteria to deliver vaccines and cytokines (designer probiotics) has been tested.[73,74] Various strain-specific probiotics validated in the treatment of different types of diseases are listed in Table 1. Also, strains of bacteria have been genetically engineered to secrete immunomodulators, which have the potential to favorably influence the immune system.[75,76] An overall summary of the various applications and recent advances in the field of probiotics with regard to enhancing human health is summarized in Figure. 1.

There is a growing interest in the area of probiotics in the recent decade based on the "hygiene hypothesis" stating that modern living conditions can lead to defective maturation and diminished immune regulation, leading to inappropriate immune responses. The "old friends" (probiotic bacteria) hypothesis has evolved as a result into a concept that lies behind attempts to modulate disease by altering the gut flora, thereby enhancing human health. The efficacy and the beneficial effects of symbionts on human health are strain- and dose-dependent. Also, normalization of the unbalanced indigenous microflora of the intestinal tract by ingestion of specific strains of the healthy microflora forms the rationale of probiotic therapy. Evidence is also emerging for the use of probiotics in the prevention of irritable bowel syndrome and in ulcerative colitis, Crohn's disease, and various other non-gastrointestinal diseases such as vaginal and oral health and cancer.[77] The capacity to assess the gut microbiota has expanded dramatically with the advent of molecular techniques.[78] This may also facilitate the potential use of genetically modified probiotic bacteria for pharmaceutical uses. Many studies reported so far have important methodological limitations, making it difficult to draw unequivocal conclusions regarding efficacy, dose, duration of treatment, etc. Therefore, the reports and conclusions obtained from those results should always be viewed with some skepticism. Also, considerable differences exist in composition, doses, and biological activity between various commercial preparations, and one consistent feature is that not all probiotic bacteria have similar therapeutic effects. Some of the effects of probiotics are beginning to be understood at a molecular level, and as a result of new gene technologies, an increased understanding of probiotics is emerging; further research requires extensive experimental validation and clinical research to be done in this area.[79,80] For example, a novel mechanism of cytoprotection by p40, a soluble product of *Lactobacillus rhamnosus* GG, mediated via epidermal growth factor receptor indicates tremendous therapeutic potential in three models of chemically induced colitis.[81] Also, cooperation between medical health professionals/clinicians, microbiologists, immunologists, gastroenterologists, and nutritionists is required, reflecting the multidisciplinary nature of the probiotic field in the area of functional food research. Many lines of evidence suggest that the potential use of probiotics for therapeutic interventions has identified patient populations that benefit from the approach with regard to specific illnesses and disorders. However, with the complex nature of immune response and the interaction between symbionts and the human GI tract, further research will be needed to provide better understanding for the field.

AUTHOR INFORMATION

Corresponding Author

*Phone: (519) 824-4120, ext. 52901. Fax: (519) 824-6631. E-mail: ymine@uoguelph.ca.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Eckburg, P. B.; Bik, E. M.; Bernstein, C. N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S. R.; Nelson, K. E.; Relman, D. A. Diversity of the human intestinal microbial flora. *Science* **2005**, *308*, 1635−1638.

(2) Gill, S. R.; Pop, M.; Deboy, R. T.; Eckburg, P. B.; Turnbaugh, P. J.; Samuel, B. S.; Gordon, J. I.; Relman, D. A.; Fraser-Liggett, C. M.; Nelson, K. E. Metagenomic analysis of the human distal gut microbiome. *Science* **2006**, *312*, 1355−1359.

(3) Mazmanian, S. K.; Kasper, D. L. The love−-hate relationship between bacterial polysaccharides and the host immune system. *Nat. Rev. Immunol.* **2006**, *6*, 849−858.

(4) Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K. S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; Mende, D. R.; Li, J.; Xu, J.; Li, S.; Li, D.; Cao, J.; Wang, B.; Liang, H.; Zheng, H.; Xie, Y.; Tap, J.; Lepage, P.; Bertalan, M.; Batto, J. M.; Hansen, T.; Le Paslier, D.; Linneberg, A.; Nielsen, H. B.; Pelletier, E.; Renault, P.; Sicheritz-Ponten, T.; Turner, K.; Zhu, H.; Yu, C.; Li, S.; Jian, M.; Zhou, Y.; Li, Y.; Zhang, X.; Li, S.; Qin, N.; Yang, H.; Wang, J.; Brunak, S.; Doré, J.; Guarner, F.; Kristiansen, K.; Pedersen, O.; Parkhill, J.; Weissenbach, J.; Meta HIT Consortium; Bork, P.; Ehrlich, S. D.; Wang, J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **2010**, *464*, 59−65.

(5) Ley, R. E. M.; Hamady, C.; Lozupone, P. J.; Turnbaugh, R. R.; Ramey, R. R.; Bircher, J. S.; Schlegel, M. L.; Tucker, T. A.; Schrenzel, M. D.; Knight, R.; Gordon, J. I. Evolution of mammals and their gut microbes. *Science* **2008**, *320*, 1647−1651.

(6) Penders, J.; Stobberingh, P. A.; Brandt, V. D.; Thijs, C. The role of intestinal microbiota in the development of atopic disorders. *Allergy* **2007**, *62*, 1223−1236.

(7) Hanson, L. A. Feeding and infant development breast-feeding and immune function. *Proc. Nutr. Soc.* **2007**, *66*, 384−396.

(8) Sansonetti, P. J.; Medzhitov, R. Learning tolerance while fighting ignorance. *Cell* **2009**, *138*, 416−420.

(9) Ley, R. E.; Hamady, M.; Lozupone, C.; Turnbaugh, P. J.; et al. Evolution of mammals and their gut microbes. *Science* **2008**, *320*, 1647−1651.

(10) Ng, S. C.; Hart, A. L.; Kamm, M. A.; Stagg, A. J.; Knight, S. C. Mechanisms of action of probiotics: recent advances. *Inflamm. Bowel Dis.* **2009**, *15*, 300−310.

(11) Vanderpool, C.; Yan, F.; Polk, D. B. Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. *Inflamm. Bowel Dis.* **2008**, *14*, 1585−1596.
(12) Phillips, M.; Kailasapathy, K.; Tran, L. Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in cheddar cheese. *Int. J. Food Microbiol.* **2006**, *108*, 276−280.
(13) Adhikari, K.; Mustapha, A.; Grun, I. U. Survival and metabolic activity of microencapsulated *Bifidobacterium longum* in stirred yogurt. *J. Food Sci.* **2003**, *68*, 275−280.
(14) Homayouni, A.; Ehsani, M. R.; Azizi, A.; Razavi, S. H.; Yarmand, M. S. Growth and survival of some probiotic strains in simulated ice cream conditions. *J. Appl. Sci.* **2008**, *8*, 379−382.
(15) Homayouni, A.; Ehsani, M. R.; Azizi, A.; Razavi, S. H.; Yarmand, M. S. Spectrophotometrically evaluation of probiotic growth in liquid media. *Asian J. Chem.* **2008**, *20*, 2414−2420.
(16) Wen, L.; Ley, R. E.; Volchkov, P. Y.; Stranges, P. B.; Avanesyan, L.; Stonebraker, A. C.; Hu, C.; Wong, F. S.; Szot, G. L.; Bluestone, J. A. Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature* **2008**, *455*, 1109−1113.
(17) Garrett, W. S.; Lord, G. M.; Punit, S.; Lugo-Villarino, G.; Mazmanian, S. K.; Ito, S.; Glickman, J. N.; Glimcher, L. H. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* **2007**, *131*, 33−45.
(18) Zoetendal, E. G.; Collier, C. T.; Koike, S.; Mackie, R. I.; Gaskins, H. R. Molecular ecological analysis of the gastrointestinal microbiota: a review. *J. Nutr.* **2004**, *34*, 465−472.
(19) Tlaskalova-Hogenova, H.; Stepankova, R.; Hudcovic, T.; Tuckova, L.; Cukrowska, B.; Lodinova-Zadnikova, R.; Kozakova, H.; Rossmann, P.; Bartova, J.; Sokol, D.; Funda, D. P.; Borovska, D.; Rehakova, Z.; Sinkora, J.; Hofman, J.; Drastich, P.; Kokesova, A. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol. Lett.* **2004**, *93*, 97−108.
(20) Li, M.; Wang, B. H.; Zhang, M. H.; Rantalainen, M.; Wang, S. Y.; Zhou, H. K.; Zhang, Y.; Shen, J.; Pang, X. Y.; Zhang, M. L.; Wei, H.; Chen, Y.; Lu, H. F.; Zuo, J.; Su, M. M.; Qiu, Y. P.; Jia, W.; Xiao, C. N.; Smith, L. M.; Yang, S. L.; Holmes, E.; Tang, H. R.; Zhao, G. P.; Nicholson, J. K.; Li, L. J.; Zhao, L. P. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2117−2122.
(21) Hamer, H. M.; Jonkers, D.; Venema, K.; Vanhoutvin, S.; Troost, F. J.; Brummer, R. J. Review article: the role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* **2008**, *27*, 104−119.
(22) Wong, J. M.; de Souza, R.; Kendall, C. W.; Emam, A.; Jenkins, D. J. Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* **2006**, *40*, 235−243.
(23) Hooper, L. V.; Midvedt, T.; Gordon, J. I. How host microbial interactions shape the nytrient environment of the mamamlion intestine. *Annu. Rev. Nutr.* **2002**, *22*, 283−273.
(24) Conly, J. M.; Stein, L.; Worobetz, L.; Rutledge-Harding, S. The contribution of vitamin K2 produced by the intestinal microflora to human nutritional requeriments for vitamin K. *Am. J. Gastroenterol.* **1994**, *89*, 915−923.
(25) Lupton, J. R. Microbial degradation products influence colon cancer risk: the butyrate controversy. *J. Nutr.* **2004**, *134*, 479−482.
(26) Kau, A. L.; Ahern, P. P.; Griffin, N. W.; Goodman, A. L.; Gordon, J. I. Human nutrition, the gut microbiome and immune system, envisioning the future. *Nature* **2011**, *474*, 327−336.
(27) Collado, M. C.; Isolauri, E.; Salminen, S.; Sanz, Y. The impact of probiotic on gut health. *Curr. Drug Metab.* **2009**, *10*, 68−78.
(28) Lam, E. K.; Woo, P. C.; Cho, C. H. Probiotics and gastrointestinal disorders. *Pharmacol. Online* **2005**, *1*, 88−147.
(29) Broekaert, I. J.; Walker, W. A. Probiotics and chronic disease. *J. Clin. Gastroenterol.* **2006**, *40*, 270−274.
(30) Isolauri, E. Probiotics for infectious diarrhoea. *Gut* **2003**, *52*, 436−437.
(31) Klein, K.; Stevens, R. The clinical use of probiotics for young children. *J. Fam. Health Care* **2008**, *18*, 66−68.
(32) Weizman, Z.; Asli, G.; Alsheikh, A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics* **2005**, *115*, 5−9.
(33) Hamilton-Miller, J. M. T. Probiotics in the management of irritable bowel syndrome: a review of clinical trials. *Microb. Ecol. Health Dis.* **2001**, *13*, 212−216.
(34) Borowiec, A. M.; Fedorak, R. N. The role of probiotics in management of irritable bowel syndrome. *Curr. Gastroenterol. Rep.* **2007**, *9*, 393−400.
(35) Malinen, E.; Rinttilä, T.; Kajander, K.; Mättö, J.; Kassinen, A.; Krogius, L.; Saarela, M.; Korpela, R.; Palva, A. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am. J. Gastroenterol.* **2005**, *100*, 373−382.
(36) Shanahan, F. Probiotics and inflammatory bowel disease: is there a scientific rationale? *Inflamm. Bowel Dis.* **2000**, *6*, 107−115.
(37) Gill, H.; Prasad, J. Probiotics, immunomodulation and health benefits. *Adv. Exp. Med. Biol.* **2008**, *606*, 423−454.
(38) Maslowski, K. M.; Mackay, C. R. Diet, gut microbiota and immune responses. *Nat. Immunol.* **2011**, *12*, 5−9.
(39) Borchers, A. T.; Selmi, C.; Meyers, F. J.; Keen, C. L.; Gershwin, M. E. Probiotics and immunity. *J. Gastroenterol.* **2009**, *44*, 26−46.
(40) Delcenserie, V.; Martel, D.; Lamoureux, M.; Amiot, J.; Boutin, Y.; Roy, D. Immunomodulatory effects of probiotics in the intestinal tract. *Curr. Issues Mol. Biol.* **2008**, *10*, 37−54.
(41) Casas, I. A.; Dobrogosz, W. J. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microb. Ecol. Health Dis.* **2000**, *12*, 247−285.
(42) Valeur, N.; Engel, P.; Carbajal, N.; Connolly, E.; Ladefoged, K. Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl. Environ. Microbiol.* **2004**, *70*, 1176−1181.
(43) Kirjavainen, P. V.; Apostolou, E.; Arvola, T.; Salminen, S. J.; Gibson, G. R.; Isolauri, E. Characterizing the composition of intestinal microflora as a prospective treatment target in infant allergic disease. *FEMS Immunol. Med. Microbiol.* **2001**, *32*, 1−7.
(44) Kalliomaeki, M.; Salminen, S.; Arvilomi, H.; Koskinen, P.; Isolauri, E. Probiotics in primary prevention of atopic disease. A randomised placebo-controlled trial. *Lancet* **2001**, *357*, 1076−1079.
(45) Salminen, S. J.; Gueimonde, M.; Isolauri, E. Probiotics that modify disease risk. *J. Nutr.* **2005**, *135*, 1294−1298.
(46) Suzuki, S.; Shimojo, N.; Tajiri, Y.; Kumemura, M.; Kohno, Y. Differences in the composition of intestinal Bifidobacterium species and the development of allergic diseases in infants in rural Japan. *Clin. Exp. Allergy* **2007**, *37*, 506−511.
(47) Gruber, C.; Wendt, M.; Sulser, C.; Lau, S.; Kulig, M.; Wahn, U.; Werfel, T.; Niggemann, B. Randomized, placebo-controlled trial of *Lactobacillus rhamnosus* GG as treatment of atopic dermatitis in infancy. *Allergy* **2007**, *62*, 1270−1276.
(48) Wickens, K.; Black, P. N.; Stanley, T. V.; Mitchell, E.; Fitzharris, P.; Tannock, G. W.; Purdie, G.; Crane, J. Probiotic Study Group. A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J. Allergy. Clin. Immunol.* **2008**, *122*, 788−794.
(49) Rupa, P.; Schmied, J.; Lai, S.; Wilkie, B. N. Attenuation of allergy to ovomucoid in pigs by neonatal treatment with heat-killed *Escherichia coli* or *E. coli* producing porcine IFN-γ. *Vet. Immunol. Immunopathol.* **2009**, *132*, 78−83.
(50) Rupa, P.; Schmied, J.; Wilkie, B. N. Prophylaxis of experimentally induced ovomucoid allergy in neonatal pigs using *Lactococcus lactis*. *Vet. Immunol. Immunopathol.* **2011**, *140*, 23−29.
(51) de LeBlanc, A. D. M.; Matar, C.; Perdigón, G. The application of probiotics in cancer. *Br. J. Nutr.* **2007**, *98*, S105−S110.
(52) Kumar, M.; Kumar, A.; Nagpal, R.; Mohania, D.; Behare, P.; Verma, V.; Kumar, P.; Poddar, D.; Aggarwal, P. K.; Henry, C. J.; Jain, S.; Yadav, H. Cancer-preventing attributes of probiotics: an update. *Int. J. Food Sci. Nutr.* **2010**, *61*, 473−496.

(53) Turnbaugh, P. J.; Bäckhed, F.; Fulton, L.; Gordon, J. I. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* **2008**, *3*, 213−223.
(54) Foo, N. P.; Yang, H. O.; Chiu, H. H.; Chan, H. Y.; Liao, C. C.; Yu, C. K.; Wang, Y. J. Probiotics prevent the development of 1,2-dimethylhydrazine (DMH)-induced colonic tumorigenesis through suppressed colonic mucosa cellular proliferation and increased stimulation of macrophages. *J. Agric. Food Chem.* **2011**, *59*, 13337−13345.
(55) Turnbaugh, P. J.; Ridaura, V. K.; Faith, J. J.; Rey, F. E.; Knight, R.; Gordon, J. I. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* **2009**, *1*, 1−10.
(56) Larsen, N. F. K.; Vogensen, F,K.; van den Berg, F. W.; Nielsen, D. S.; Andreasen, A. S.; Pedersen, B. K.; Al-Soud, W. A.; Sørensen, S. J.; Hansen, L. H.; Jakobsen, M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* **2010**, *5*, e9085.
(57) Luoto, R. K.; Laitinen, K.; Nermes, M.; Isolauri, E. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. *Br. J. Nutr.* **2010**, 1−8.
(58) El-Nezami, H. S.; Polychronaki, N. N.; Ma, J.; Zhu, H.; Ling, W.; Salminen, E. K.; Juvonen, R. O.; Salminen, S. J.; Poussa, T.; Mykkänen, H. M. Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from southern China. *Am. J. Clin. Nutr.* **2006**, *83*, 1199−1203.
(59) Meurman, J. H. Probiotics: do they have a role in oral medicine and dentistry? *Eur. J. Oral Sci.* **2005**, *113*, 188−196.
(60) Reid, G. Probiotic agents to protect the urogenital tract against infection. *Am. J. Clin. Nutr.* **2001**, *73*, 437s−443s.
(61) Li, J.; McCormick, J.; Bocking, A.; Reid, G. Importance of vaginal microbes in reproductive health. *Reprod. Sci.* **2012**, *19*, 235−242.
(62) Vanderpool, C.; Yan, F.; Polk, D. B. Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. *Inflamm. Bowel Dis.* **2008**, *14*, 1585−1596.
(63) Renz, H.; Brandtzaeg, P.; Hornef, M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. *Nat. Rev. Immunol.* **2011**, *12*, 9−23.
(64) Ashida, H.; Ogawa, M.; Kim, M.; Mimuro, H.; Sasakawa, C. Bacteria and host interactions in the gut epithelial barrier. *Nat. Chem. Biol.* **2011**, *8*, 36−45.
(65) Corthésy, B.; Gaskins, H. R.; Mercenier, A. Cross-talk between probiotic bacteria and the host immune system. *J. Nutr.* **2007**, *137*, 781S−790S.
(66) Foligne, B.; Nutten, S.; Grangette, C.; Dennin, V.; Goudercourt, D.; Poiret, S.; Dewulf, J.; Brassart, D.; Mercenier, A.; Pot, B. Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. *World J. Gastroenterol.* **2007**, *13*, 236−243.
(67) Spiller, R. Review article: probiotics and prebiotics in irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **2008**, *28*, 385−396.
(68) Reid, G.; Bruce, A. W. Probiotics to prevent urinary tract infections: the rationale and evidence. *World J. Urol.* **2006**, *24*, 28−32.
(69) Kukkonen, K.; Savilahti, E.; Haahtela, T.; Juntunen-Backman, K.; Korpela, R.; Poussa, T.; Tuure, T.; Kuitunen, M. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J. Allergy Clin. Immunol.* **2007**, *119*, 192−198.
(70) Pereira, D. I.; Gibson, G. R. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Crit. Rev. Biochem. Mol. Biol.* **2002**, *37*, 259−281.
(71) Lieske, J. C.; Goldfarb, D. S.; De Simone, C.; Regnier, C. Use of a probiotic to decrease enteric hyperoxaluria. *Kidney Int.* **2005**, *68*, 1244−1249.
(72) Gorbach, S. L. Probiotics and gastrointestinal health. *Am. J. Gastroenterol.* **2000**, *95*, S2−S4.
(73) Van Huynegem, K.; Loos, M.; Steidler, L. Immunomodulation by genetically engineered lactic acid bacteria. *Front. Biosci.* **2009**, *14*, 4825−4835.
(74) Steidler, L. Genetically engineered probiotics. *Best Pract. Res. Clin. Gastroenterol* **2003**, *17*, 861−876.
(75) Steidler, L.; Hans, W.; Schotte, L.; Neirynck, S.; Obermeier, F.; Falk, W.; Fiers, W.; Remaut, E. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* **2000**, *289*, 1352−1355.
(76) Vandenbroucke, K.; Hans, W.; Van Huysse, J.; Neirynck, S.; Demetter, P.; Remaut, E.; Rottiers, P.; Steidler, L. Active delivery of trefoil factors by genetically modified *Lactococcus lactis* prevents and heals acute colitis in mice. *Gastroenterology* **2004**, *127*, 502−513.
(77) MacDonald, R. S.; Wagner, K. Influence of dietary phytochemicals and microbiota on colon cancer risk. *J. Agric. Food Chem.* **2012**, *60*, 6728−6735.
(78) Rodrigues, D.; Santos, C. H.; Rocha-Santos, T. A. P.; Gomes, A. M.; Goodfellow, B. J.; Freitas, A. C. Metabolic profiling of potential probiotic or synbiotic cheeses by nuclear magnetic resonance (NMR) spectroscopy. *J. Agric. Food Chem.* **2011**, *59*, 4955−4961.
(79) Laparra, J. M.; Sanz, Y. Interactions of gut microbiota with functional food components and nutraceuticals. *Pharmacol. Res.* **2010**, *61*, 219−25.
(80) Makoto, S.; Satoshi, H. Gut as a target for functional food. *Trends Food Sci. Technol.* **2011**, *22*, 646−650.
(81) Fordjour, L.; D'Souza, A.; Cai, C.; Ahmad, A.; Valencia, G.; Kumar, D.; Aranda, J. V.; Beharry, K. D. Comparative effects of probiotics, prebiotics, and synbiotics on growth factors in the large bowel in a rat model of formula-induced bowel inflammation. *J. Pediatr. Gastroenterol. Nutr.* **2010**, *51*, 507−513.