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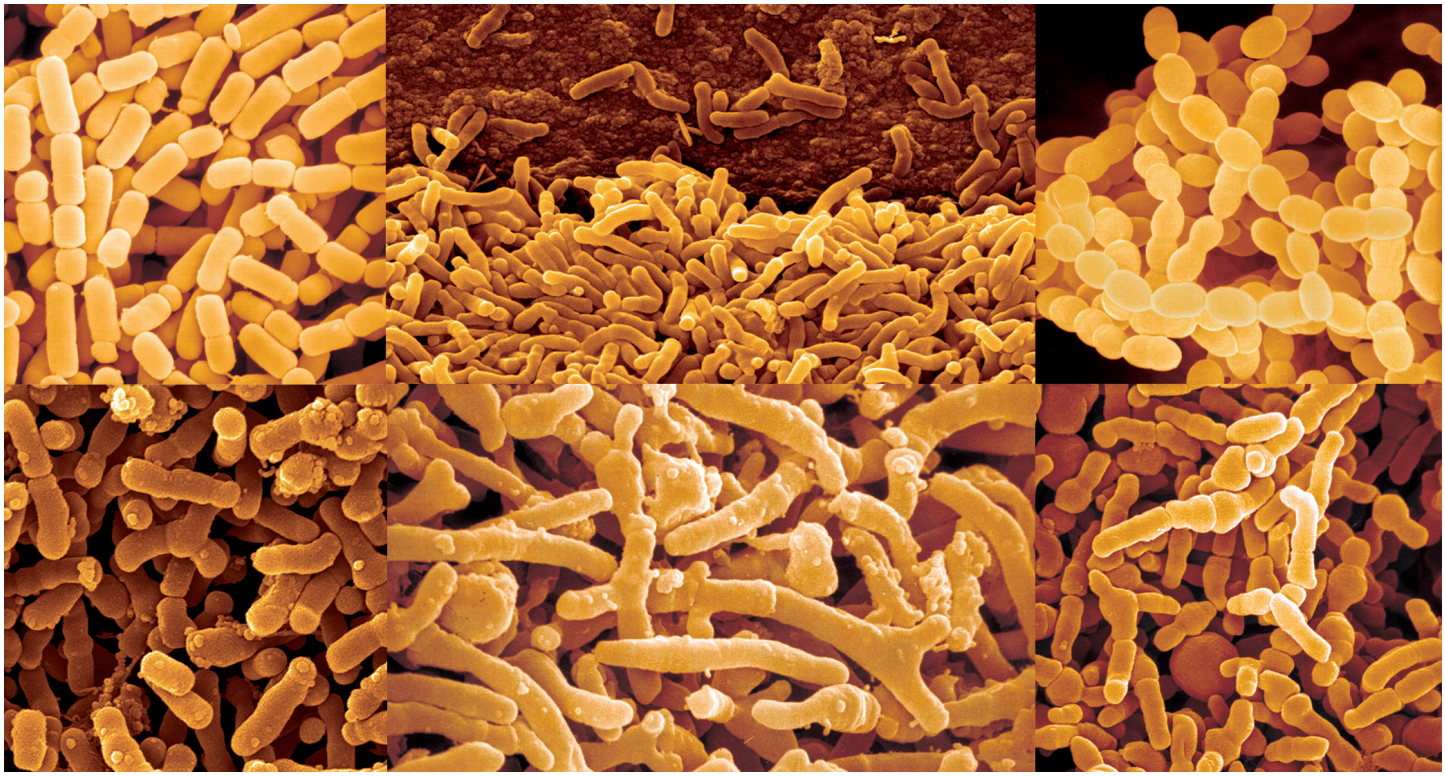
Probiotics: Their Potential to Impact Human Health



Scanning electron micrographs (clockwise from upper left) of *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Bifidobacterium breve*. (Photos courtesy of Prof. Lorenzo Morelli, Instituto di Microbiologia, Piacenza, Italy.)

Abstract

Probiotics—live microorganisms that when administered in adequate amounts confer a health benefit on the host—have been studied for both human and animal applications, and worldwide research on this topic has accelerated in recent years. This paper reviews the literature on probiotics, describes how probiotics work in human ecosystems, and outlines the impact of probiotics on human health and disease. The paper also addresses safety issues of probiotic use, suggests future developments in the field of probiotics, and provides research and policy recommendations. Product considerations and potential future developments regarding probiotics also are discussed. The authors conclude that controlled human studies have revealed a diverse range of health benefits from consumption of probiotics, due largely to their impact on immune function or on microbes colonizing the body. Additional, well-designed and properly controlled human and mechanistic studies with probiotics will advance the essential understanding of active principles, mechanisms of action, and degree of effects that can be realized by specific consumer groups. Recommendations include establishment of a standard of identity for the term "probiotic," adoption of third-party verification of label claims, use of probiotics selectively in clinical conditions, and use of science-based assessment of the benefits and risks of genetically engineered probiotic microbes.

Introduction

Probiotics[1] are live microorganisms that when administered in adequate amounts confer a health benefit on the host (UNFAO/WHO 2001). Probiotics commonly are isolated from human and animal intestinal tracts. Dead bacteria, products derived from bacteria, or end products of bacterial growth also may impart certain benefits, but these derivatives are not considered to be probiotics because they are not alive when administered. Native bacteria are not probiotics until the bacteria are isolated, purified, and proved to have a health benefit when administered. Probiotics have been

[1] Italicized terms (except genus and species names) are defined in the Glossary.

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Table 1. Key genera and species of microbes studied and used as probiotics

Genus	Species
Lactobacillus	*acidophilus* *brevis* *delbrueckii*[a] *fermentum* *gasseri* *johnsonii* *paracasei* *plantarum* *reuteri* *rhamnosus* *salivarius*
Bifidobacterium	*adolescentis* *animalis*[b] *bifidum* *breve* *infantis* *longum*
Streptococcus	*thermophilus* *salivarius*
Enterococcus	*faecium*
Escherichia	*coli*
Bacillus	*coagulans*[c] *clausii*
Saccharomyces	*cerevisiae*[d]

[a] *L. delbrueckii* subsp. *bulgaricus* is typically used as a starter culture for yogurt.

[b] A subspecies of *B. animalis* is *B. animalis* subsp. *lactis*. Several commercial probiotic strains are members of this subspecies and commonly are referred to as *B. lactis*, although the correct designation is *B. animalis* subsp. *lactis*.

[c] Some manufacturers market a strain they call "Lactobacillus sporogenes." The microbe marketed under this name is likely a *Bacillus coagulans*, not related to the true *Lactobacillus* genus (Sanders, Morelli, and Bush 2001).

[d] A prominent probiotic strain marketed as "Saccharomyces boulardii" (not a valid species name) is a strain of *Saccharomyces cerevisiae*.

studied for both human and animal applications. Key microbial species used as human probiotics are listed in Table 1.

Worldwide, a diverse array of probiotic products is on the market. Yogurt is perhaps the most common probiotic-carrying food, but the market has expanded beyond yogurt. Cheese, fermented and unfermented milks, juices, smoothies, cereal, nutrition bars, and infant/toddler formula all are food vehicles for probiotic delivery. In addition to being sold as foods, probiotics are sold as dietary supplements, medical foods, and drugs (although there are no probiotics currently sold as drugs in the United States). Often these products are composed of concentrated, dried microbes packaged into capsules, tablets, or sachets. This format is convenient for the delivery of large numbers of microbes that, if manufactured and stored properly, can be quite stable even at room temperature.

What motivates people to choose one format over another has much to do with personal preference, product availability in different geographic regions, or individual needs, although fewer types of probiotic foods are available in the United States than in parts of Europe or Asia. Product formulation may impact greatly how a probiotic survives during product storage and if it reaches the target site in the body physiologically capable of exerting its benefits. Furthermore, additive or even synergistic activities of components in addition to probiotics in the product may enhance the product's health-promoting properties. In the end, each specific product must be judged based on its ability to deliver health benefits through a well-formulated probiotic content.

Researchers have studied many possible benefits of probiotics (Table 2), and the pace of research in recent years has accelerated. More than four times the number of human clinical trials on probiotics were published during the period from 2001 to 2005 than from 1996 to 2000. To the uninitiated, the list of benefits from probiotics seems too diverse to be possible. But once it is understood that probiotics can impact any colonized regions of the body and that bacteria have the potential to influence the body both *locally* and *systemically*, the scope of benefits can be appreciated.

The authors of this Issue Paper have reviewed the literature on probiotics encompassing both U.S. and international research reports. In this paper, the authors describe the characteristics of probiotics, discuss what is known about the microbes that colonize humans, and outline the impacts of probiotics on human health and disease for specific conditions. The paper also addresses the regulatory status of probiotic foods in the United States, suggests future developments in the field of probiotics, and provides research and policy recommendations. A detailed discussion of "How Probiotics Work" can be found in Appendix 1.

Characteristics of Probiotics

Certain physiological characteristics may be important for probiotics targeted toward particular applications. For

Table 2. Targets for research on probiotics, with some example references

Health Target	Reference[a]
Immune enhancement	Gill and Guarner 2004
Diarrhea (rotavirus, travelers', antibiotic-associated, *C. difficile*)	Szajewska, Ruszczynski, and Radzikowski 2006; McFarland 2006; Sazawal et al. 2006
Alteration of intestinal microbiota	Agence Francaise de Sécurité Sanitaire des Aliments/ French Food Safety Agency (AFSSA) 2005
Harmful intestinal microbe activities	Agence Francaise de Sécurité Sanitaire des Aliments/ French Food Safety Agency (AFSSA) 2005
Lactose digestion	Marteau et al. 1990
Allergy development and symptoms	Kalliomaki et al. 2001; Viljanen et al. 2005
Inflammatory bowel diseases	Gionchetti et al. 2000; Kruis et al. 2004
Vaginal infections	Anukam et al. 2006 a, b
Delivery of cloned components active in gut (IL10, vaccines, anti-viral agents, toxin receptors)	Braat et al. 2006
H. pylori colonization of the stomach	Sheu et al. 2006
Absences from work, daycare	Tubelius, Stan, and Zachrisson 2005; Weizman, Asli, and Alsheikh 2005
Irritable bowel syndrome	O'Mahony et al. 2005
Colds	de Vrese et al. 2005
Growth for undernourished young children	Saran, Gopalan, and Krishna 2002
Colon tumors (primary evidence in animals)	Ishikawa et al. 2005
Dental caries	Nase et al. 2001
Blood pressure	Jauhiainen et al. 2005
Blood lipid profiles	Hlivak et al. 2005

[a]For additional information, see www.usprobiotics.org

example, resistance to stomach acid and *pancreatic secretions* such as bile and digestive enzymes would be important for probiotics needing to survive in high numbers through the small intestine. But if the target site for the probiotic is, for example, the mouth, these traits would not be relevant. It is apparent from the broad range of potential probiotic targets that what is required of a probiotic depends on the specific target function. Yet some basic criteria for probiotics can be set: namely, that probiotics

- must be shown to exert a beneficial effect on the consumer, preferably with a mechanistic explanation of how this occurred;
- are nonpathogenic, nontoxic, and free of significant adverse side effects;
- retain stability during the intended shelf life of the product;
- contain an adequate number of viable cells to confer the health benefit;
- are compatible with product format to maintain desired sensory properties; and
- are labeled in a truthful and informative manner to the consumer.

It is generally accepted that health claims have to be supported by well-conducted clinical trials in the targeted population. But the selection of *strains* from the huge natural reservoir of candidates, the characterization of the purified strain, and the substantiation of a physiological benefit in humans comprise a long path unique to the intended use for the strain. This path is complicated by the lack of rigorous validation of the different laboratory methods conventionally used to select and characterize probiotic candidates.

Human-Associated Microbial Ecosystems

The microbes that colonize humans are dynamic components of the body's ecosystem, both gaining and providing nourishment. In the process, these microbes aid in the development of intestinal cells and participate in the maturation and function of the *innate immune system*. Although much is still unknown about the microbes that colonize humans, some important points can be made.

- Each individual has his or her own unique population of microbes, even if there are commonalities of *species* among people.
- The microbes colonizing different regions of the human body (skin, mouth, gastrointestinal tract, vaginal tract of women) are both diverse and numerous, and they differ according to their habitat.
- Intestinal microbes are fairly stable through time, although transitions occur at weaning and again in the elderly. Colonizing *microbiota* can be impacted by antibiotics, diet, immunosuppression, intestinal cleansing, and other factors; however, the populations generally return to normal after being disturbed, with no intervention.
- Most colonizing microbes are not harmful in their natural body habitat, but some may generate undesirable metabolic end products.
- The composition of the "normal, healthy intestinal microbiota" is not defined currently. Likewise, the characteristics of the intestinal microbiota that may lead to many different disease states also are not well understood. The nature of the end products of growth of these microbes may be as important as which specific microbes are present.
- Activities such as regulating immune function, enhancing the intestinal barrier to prevent un-

wanted microbes from entering the blood stream, *colonization resistance*, and digestion are important functions of colonizing microbes.

Regions of the Human Body Colonized by Microbes

Bacteria are prevalent in several regions of the body, including the mouth, nose, pharynx, intestinal tract, vaginal tract, and skin (Willis et al. 1999) (See Figure 1). The stomach is not heavily colonized because of its low pH, and typically harbors up to 10^3 *colony forming units* (CFU) per gram of contents, mainly consisting of lactobacilli, streptococci, and yeasts (Holzapfel et al. 1998). In addition, *Helicobacter pylori* colonization of the stomach is endemic in certain geographical regions of the world. The duodenum, or first part of the small intestine, also has low microbial populations because of both the quick transit of contents through it and the presence of pancreatic secretions that create a hostile environment for microbes. There is a progressive increase in both numbers and species of microbes, however, along the *jejunum* and *ileum*, from approximately 10^4 to 10^{6-7} CFU per gram of contents at the ileo-cecal region (Salminen et al. 1998). The colon is the most heavily populated area of the gastrointestinal tract, with numbers typically in the region of 10^{11} CFU per gram (wet weight) of contents. This environment supports greater bacterial growth with a slower *transit time*, ready availability of nutrients, and favorable pH.

Recently, Eckburg and colleagues (2005) characterized the microbiota of feces and the intestinal lining of the colon of three healthy humans and found that the majority of microbes present were not from species that have been cultured to date. These authors concluded that additional research still is needed to understand fully the microecology of the intestine.

In women, microbes (many of fecal origin) inhabit the vagina to a concentration of approximately 10^{7-8} CFU per milliliter (ml) of fluid, with *Lactobacillus* species dominant in healthy subjects and urinary and vaginal pathogens dominant in patients with infection.

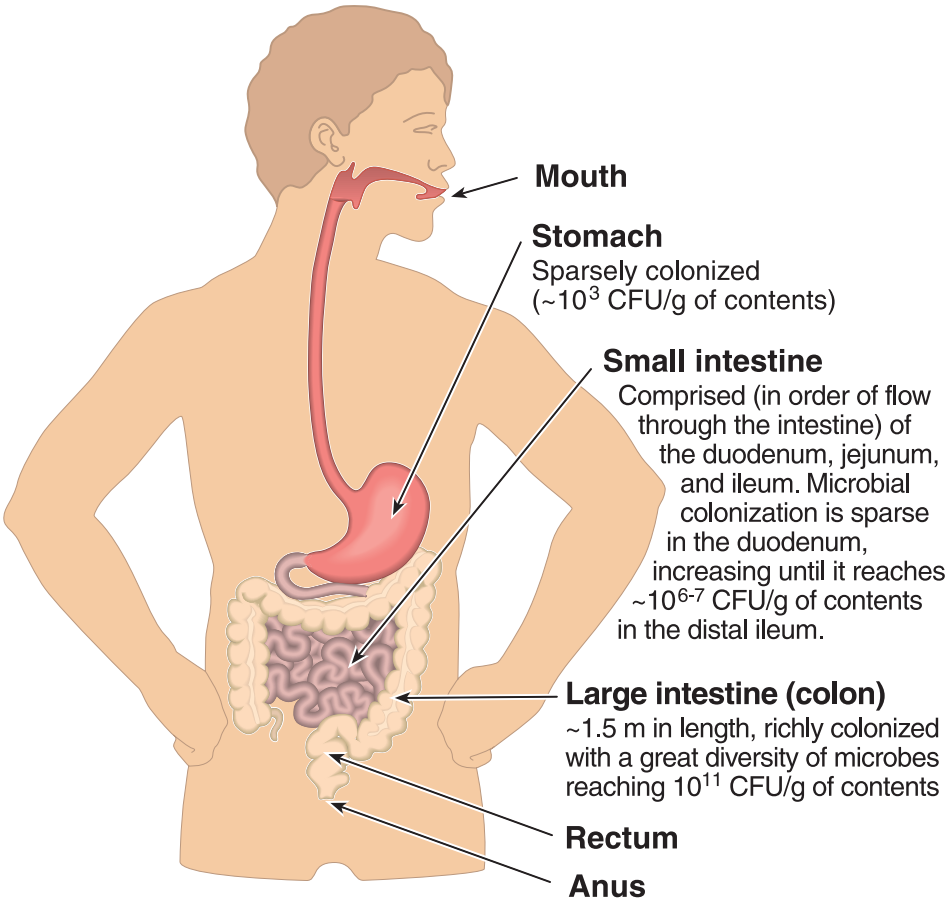

Figure 1. The human gastrointestinal tract.

Probiotic Impact on Colonizing Microbiota

The probiotic concept asserts that adding the right live microbes to this complex system can result in physiological benefits. In some instances, these effects may result from alteration of the population or activities of colonizing microbes. In other cases, effects may be due to direct interaction of the probiotic with host cells. The gut remains the most studied site of action for probiotics. Most human studies with probiotics have targeted specific health benefits associated with the gut microbiota, and a few studies have targeted the stomach, mouth, throat, or vagina.

Several laboratory scale models have been developed to simulate the intestine (Macfarlane, Macfarlane, and Gibson 1998; Rumney and Rowland 1992). These models are useful for estimating probiotic impact on microbial populations or biochemical markers, probiotic survival during transit, and strain-specific effects. But they cannot fully mimic host factors, and the effects ultimately must be measured in the target host.

Analysis of fecal samples for the presence of probiotics that have been consumed is used often as a proxy measure of probiotic function. The rationale is "If they make it all the way through the body alive, they can do some good." Although easily obtained, fecal samples cannot be relied on as accurate indicators of the microecology upstream. If fed in high enough numbers, most probiotic strains can be recovered from feces. This observation is often termed *colonization*, but commonly it is not differentiated from simple transient passage. A more precise word to describe this situation is persistence, assessed by the number of organisms present over time in feces following a feeding period. Persistence would be expected to be a function of the time it takes the probiotic to travel through the *alimentary canal*, as well as death and growth

rates of the probiotic in the body.

Discriminating the fed strain from naturally occurring members of the same *genus* or species in the gut can be a challenge. *Culture methodologies* frequently are not discriminatory enough for this type of differentiation unless the probiotic has a unique physiological or biochemical trait that allows it to be distinguished from background microbes. It is possible, however, to apply modern deoxyribonucleic acid (DNA)-based methods to discriminate between fed and naturally occurring strains (McCartney 2002). But the significance of fecal recovery of probiotics must be interpreted carefully because there is no known clinical benefit associated with higher populations of lactobacilli or bifidobacteria recovered in feces.

There also is debate about the importance of any probiotic-induced changes observed in gut microbiota. The most consistently observed change is the increase in the populations of the genus of the fed probiotic strain. Probiotic-induced changes in the populations of more dominant intestinal groups (such as *Bacteroides, Clostridium*, or *Enterobacteriaceae*) vary from study to study and probiotic to probiotic. Several factors complicate interpretation of these findings. High intra-subject and day-to-day variability and a lack of understanding of the composition of the microbial community vertically and horizontally within the alimentary canal make it difficult to understand the significance of such findings. Perhaps more important than the microbe population changes are the resulting alterations in biochemistry and physiology of the intestine.

Probiotic Impact on Human Health and Disease

Reviews on the impact of probiotics on human health and disease are numerous and have emphasized different components of the field, such as use of probiotics in medical practice (Montrose and Floch 2005; Picard et al. 2005), use in pediatric populations (Saavedra 2007), immunomodulation (Galdeano et al. 2007), and intestinal diseases (O'Hara and Shanahan, 2007; Sheil et al. 2007). The following discussion highlights specific areas of probiotic intervention in human health and disease.

Keeping Healthy People Healthy

Early research evaluating probiotics in humans focused on relieving intestinal distress, frequently with subjects suffering from an intestinal infection or antibiotic-associated complications. As this product concept developed further, the value of probiotics to prevent, rather than treat, disease was appreciated more fully. Toward this end, studies have been conducted in healthy populations, with end points such as decreasing the incidence of colds (de Vrese et al. 2005), winter infections (Turchet et al. 2003), or even absences from work (Tubelius, Stan, and Zachrisson 2005) or day care (Weizman, Asli, and Alsheikh 2005). These controlled human studies provide support that certain probiotic strains consumed as part of a daily diet will increase the number of illness-free days. Infants were helped by *Lactobacillus reuteri,* which decreased crying time due to colic (Savino et al. 2007).

Lactose Maldigestion

Lactose is a sugar found in milk, composed of a glucose molecule linked to a galactose molecule. Lactose can be split into glucose and galactose by lactase, an enzyme produced by infants, children, and some adults. Most humans, however, quit producing this enzyme in childhood. If these people consume *dairy products with lactose*, they can develop gastrointestinal symptoms such as abdominal bloating, pain, flatulence, and diarrhea. This situation is found in 5 to 15% of adults in Northern European and American countries and in 50 to >90% of adults in African, Asian, and South American countries (de Vrese et al. 2001). These people tend to eliminate milk and dairy products from their diet, and consequently, their calcium intake may be compromised. The bacteria used as starter cultures in yogurt (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) also produce lactase, and when consumed with dairy products can improve lactose digestion and symptoms in these individuals (Kolars et al. 1984). A number of studies have demonstrated better lactose digestion, as well as a decrease in gastrointestinal symptoms, in people with this condition who consume yogurt with live cultures (deVrese et al. 2001).

Bowel Transit

Daily consumption of one to three servings of fermented milk containing a probiotic strain, *Bifidobacterium animalis* DN-173 010, decreased the amount of time it took food to travel from the mouth to the anus for people who had longer-than-desired transit time (Marteau et al. 2002). The effect was more pronounced in elderly subjects and in women. A mixture of eight different strains of lactobacilli, bifidobacteria, and *S. thermophilus* (product name, VSL#3) had no effect on gastrointestinal transit time in *irritable bowel syndrome* (IBS) subjects (Kim et al. 2003). A recent controlled study showed that *L. rhamnosus* Lcr35 improved symptoms of constipation in children (Bu et al. 2007).

Irritable Bowel Syndrome

Symptoms of abdominal pain, bloating, and flatulence commonly occur in patients with IBS. These symptoms may result in part from fermentations taking place in the colon that generate gas. Certain gut bacteria process leftover food that reaches the colon without producing gas. Other species even may consume gas, particularly hydrogen. But others produce gas, which is eliminated from the body through flatulence. In a *double-blind, clinical trial* of 48 patients with bloating-predominant IBS, the probiotic mixture VSL#3 decreased flatulence scores (Kim et al. 2005). Likewise, two other placebo-controlled trials have shown relief of abdominal bloating in patients with IBS treated with VSL#3 or *Lactobacillus plantarum* 299V (Kim et al. 2003; Nobaek et al. 2000). In children, *L. rhamnosus* GG decreased perceived abdominal distension but not abdominal pain (Bausserman and Michail 2005). Finally, two large studies in adults showed that either *Bifidobacterium infantis* 35624 or a strain mixture (*L.*

rhamnosus GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99, and *Propionibacterium freudenreichii* subsp. *shermanii* JS) can be effective in alleviating symptoms of IBS (Kajander et al. 2005; O'Mahony et al. 2005).

Some probiotics seem useful for controlling the symptoms related to intestinal gas in this group of subjects. But the number of studies is small, and further focused research is needed.

Gastrointestinal Infections

A number of clinical trials have tested the efficacy of probiotics in the prevention of acute diarrhea, including antibiotic-associated diarrhea. Both the short- and long-term use of antibiotics can produce diarrhea, particularly when multiple drugs are used. Probiotics given along with antibiotic therapy have been shown to decrease the incidence of antibiotic-associated diarrhea in children and in adults. Different strains have been tested including *L. rhamnosus* GG, the yeast *Saccharomyces cerevisiae* (boulardii) Lyo, and undefined strains of *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *bulgaricus*. *Meta-analysis* of controlled trials concluded that probiotics, particularly *L. rhamnosus* GG and *S. cerevisiae* (boulardii) Lyo, can be used to prevent antibiotic-associated diarrhea (McFarland 2006; Sazawal et al. 2006). An important complication of antibiotic treatment can be the establishment of *Clostridium difficile* infection resulting in pseudomembranous colitis. Hickson and colleagues (2007) demonstrated that a fermented milk containing *Lactobacillus casei* DN-114 001, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* can decrease the incidence of antibiotic-associated diarrhea and *C. difficile*-associated diarrhea, providing evidence for the value of this treatment for this potentially refractory condition.

Probiotics are useful as treatment of acute infectious diarrhea in children. Different strains, including *L. reuteri* SD2112, *L. rhamnosus* GG, *L. casei* DN-114 001, and *S. cerevisiae* (boulardii) Lyo, tested in controlled clinical trials decreased the severity and duration of diarrhea. Meta-analysis concludes that these probiotics are safe and effective (Szajewska, Ruszczynski, and Radzikowski 2006). Oral administration of probiotics shortens the duration of acute diarrhea in children by approximately 1 day.

Prophylactic use of probiotics has proved useful for the prevention of acute diarrhea in infants admitted to the hospital with chronic disease. Supplementation of an infant formula with *B. animalis* Bb12 and *S. thermophilus* TH4 significantly decreased the incidence of diarrhea in hospitalized infants aged 5 to 24 months (Saavedra et al. 1994). A placebo-controlled double-blind study in infants aged 1 to 36 months showed similar results for *L. rhamnosus* GG (Szajewska et al. 2001). In these two studies, control subjects were more than four times more likely to develop diarrhea than those treated with probiotics.

Several studies have investigated probiotics in the prevention of travellers' diarrhea in adults, but methodological deficiencies, such as low compliance with the treatment and problems in the follow-up, limit the validity of their conclusions (Marteau, Seksik, and Jian 2002). This topic needs further study.

Probiotics have been tested as a strategy for eradication of *H. pylori* infection of the stomach. Some strains of lactic acid bacteria are known to inhibit the growth of *H. pylori* in laboratory experiments. But results in human studies with different probiotics are mixed. Eradication of *H. pylori* was attempted by feeding yogurt containing probiotic strains selected for their ability to inhibit *H. pylori* in laboratory studies. The strategy was not effective in patients not undergoing simultaneous antibiotic therapy (Wendakoon, Thomson, and Ozimek 2002). In contrast, other clinical studies have tested the use of probiotics as a supplement to antibiotic therapy for *H. pylori* eradication. In these studies, the use of probiotics decreased the side effects of antibiotics, improved patient compliance with taking the prescribed therapy, and increased the rate at which *H. pylori* was eradicated (Lionetti et al. 2006; Myllyluoma et al. 2005; Sheu et al. 2006; Sykora et al. 2005).

Prevention of Systemic Infections

Bacterial translocation is the passage of bacteria through the lining of the intestine, which can lead to infection of organs or the blood. This passage can occur when patients have undergone surgical procedures or are seriously ill with critical conditions, such as severe acute pancreatitis, advanced liver cirrhosis, or multisystem organ failure. Probiotic organisms rarely translocate, even through a disturbed epithelium (Daniel et al. 2006).

In a study of patients with severe acute pancreatitis, treatment with *L. plantarum* 299V significantly decreased the incidence of infection (Olah et al. 2002). In another study, liver transplant patients received a synbiotic preparation (including four probiotic strains and four fermentable fibers) or a placebo consisting of the four fibers only. Postoperative infection occurred in only one patient in the treatment group of 33 compared with 17 of 33 in the placebo group (Rayes et al. 2005). The difference was highly significant. But another clinical study performed with patients who submitted to elective abdominal surgery found no effect of synbiotic treatment (four bacteria strains plus *oligofructose*) on prevention of postoperative infections (Anderson et al. 2004). In that trial, synbiotic treatment after surgery was delayed until patients were able to tolerate oral nutrition. In contrast, the liver transplant studies introduced synbiotic therapy by naso-gastric tube immediately after surgery.

Necrotizing enterocolitis resulting from immaturity and poor function of the gut mucosal barrier is a severe clinical condition that may occur in low birth weight premature infants. Two controlled studies have demonstrated that the use of probiotic mixtures in these infants significantly decreases the incidence and severity of necrotizing enterocolitis and prevents death (Bin-Nun et al. 2005; Lin et al. 2005). Data from these studies represent one of the few examples of probiotics improving survival rates. Currently, few other strategies have proved effective in decreasing the incidence, morbidity, and mortality of necrotizing enterocolitis in preterm infants.

Inflammatory Bowel Diseases

Ulcerative colitis, *pouchitis*, and *Crohn's disease* are chronic conditions

of unknown cause characterized by persistent inflammation of the intestine. Evidence suggests that abnormal activation of the *mucosal immune system* against the gut microbiota is the key event that triggers this abnormal inflammatory response that in turn causes ulcers in the gut that fail to heal, leading to chronic intestinal disease. Three studies investigated the effectiveness of an oral preparation of *Escherichia coli* Nissle 1917 compared with mesalazine, the standard treatment for maintenance of remission in patients with ulcerative colitis (Kruis et al. 1997, 2004; Rembacken et al. 1999). These studies concluded that this strain was as effective as mesalazine in maintaining remission. A small pilot study suggested that a fermented milk containing *B. breve* strain Yakult, *Bifidobacterium bifidum* strain Yakult, and *L. acidophilus* (undefined strain) can be useful to induce remission in ulcerative colitis patients with mild disease (Kato et al. 2004).

The VSL#3 probiotic mixture proved highly effective for maintaining remission of chronic relapsing pouchitis (Gionchetti et al. 2000; Mimura et al. 2004). Treatment with VSL#3 also is effective in the prevention of the onset of pouchitis after surgery to form the pouch (Gionchetti et al. 2003). In Crohn's disease, however, clinical studies with *L. rhamnosus* GG failed to show efficacy in preventing postoperative recurrence of the disease (Prantera et al. 2002) or as maintenance therapy (Bousvaros et al. 2005).

Allergy

Atopic diseases are caused by exaggerated or imbalanced immune responses to environmental and harmless antigens (allergens). The prevalence of allergic diseases in western societies is increasing at an alarming rate.

The effectiveness of *L. rhamnosus* GG in the prevention of atopic dermatitis has been reported in randomized, controlled trials (Kalliomaki et al. 2001, 2003). In a subsequent study, this same strain was combined with three other probiotic strains, *L. rhamnosus* LC705, *B. breve* Bb99, and *P. freudenreichii* subsp. *shermanii* JS, and a *prebiotic* to determine the impact on the cumulative incidence of allergic diseases (Kukkonen et al. 2007). This large study (925 subjects tracked through the 2-year follow-up) showed no effect on incidence of all allergic diseases, but the treatment did significantly prevent atopic eczema. Furthermore, several well-designed studies have provided evidence that specific strains of probiotics can be somewhat effective in treatment of established atopic dermatitis (Rosenfeldt et al. 2003; Viljanen et al. 2005; Weston et al. 2005). Effectiveness in the management of cow's milk allergy in children is associated with the use of probiotics (Kirjavainen, Salminen, and Isolauri 2003). One study, however, failed to find any effect for allergic infants with *L. rhamnosus* GG treatment for 3 months (Brouwer et al. 2006), emphasizing the importance of confirmatory studies.

Colon Cancer

Several experimental animal studies clearly demonstrated a protective effect of prebiotics such as oligofructose, probiotics such as some *Lactobacillus* and *Bifidobacterium* strains, or the combination of prebiotics and probiotics on the establishment, growth, and metastasis of transplantable and chemically induced tumors. Human intervention trials to confirm these animal studies are intrinsically difficult because of the natural history of the disease (difficulty in selecting subjects at high risk and requirement of long-term follow-up). A 4-year study of 398 subjects found that *L. casei* Shirota decreased the recurrence of atypical colonic polyps (Ishikawa et al. 2005). The European Union (EU)-sponsored "Synbiotics and Cancer Prevention in Humans" project tested a synbiotic (oligofructose plus *L. rhamnosus* GG and *B. animalis* subsp. *lactis* Bb12) in patients at risk for colonic polyps. Among several intermediate end points that were used as biomarkers of colon cancer risk, the study found that the synbiotic decreased uncontrolled growth of intestinal cells (Van Loo et al. 2005).

Vaginal Infection

Vaginal infections are caused mostly by fecal microbes ascending into the vaginal tract and displacing the normal lactobacilli microbiota. The potential of using probiotic lactobacilli to decrease the risk of bacterial or yeast vaginal infections or to improve the clinical outcome during treatment for these infections has captured the interest of researchers for decades (Reid and Bocking 2003). Until recently, most studies have been small and in need of confirmation. After preliminary assessment to document that *L. rhamnosus* GR-1 and *L. reuteri* RC-14 administered in milk could pass through the intestine, ascend to the vagina, and restore a normal lactobacilli microbiota in women prone to infections (Reid et al. 2001), these strains were delivered in yogurt to African women with bacterial vaginosis and shown to improve therapeutic outcome (Anukam et al. 2006 a, b). These studies have provided the best evidence to date for successful probiotic intervention to improve vaginal health. Some other recent studies have not shown positive results (Eriksson et al. 2005), highlighting the importance of use of effective strains and delivery systems.

Safety Considerations

As probiotics increase in popularity, it is prudent to consider whether there are any safety concerns associated with the resulting increased exposure to live microbes. It is tempting to presume that the question of safety is only about the probiotic strain being used; in fact, how the strain is used and who is consuming it are important considerations as well. For example, a safety assessment of a strain used in yogurt at 10^9 CFU/serving would be different for the same strain administered at 10^{12} CFU/*enteric coated* capsule. A safety assessment for a strain administered intravaginally would again be different. A safety assessment, therefore, must consider the nature of the specific microbe being consumed, how it is prepared, how it is administered, what dose is delivered, and the health status of the consumer.

Assessing the Safety of Probiotics

The key components of assessing safety include (1) identifying the probi-

otic using the best genetic and physiological techniques to the strain level; (2) determining the antibiotic resistance profile, including an evaluation of the likelihood that any antibiotic resistance traits would be transferred to members of the colonic microbiota; (3) if applicable, establishing a history of safe use based on the intended use of the species in question; and (4) conducting toxicity or pathogenicity assessments in validated laboratory or animal models that are relevant to the species being considered, as needed.

Most probiotic-containing food products use lactic acid bacteria that have a long history of safe use in foods. As long as the strain is devoid of any transferable antibiotic resistance genes, members of the genera *Lactococcus*, *Lactobacillus*, and *Bifidobacterium* are considered safe; infections in humans by these genera are extremely rare. There have been 180 cases of lactobacillemia and 6 cases of bifidobacteremia reported during the past 30 years (Borriello et al. 2003). All cases of bifidobacteremia occurred in subjects with abdominal infections or with obstetrical procedures or infections (Gasser 1994). There have been 69 cases of infective endocarditis attributed to lactobacilli reported during the same period. In most cases of endocarditis, dental surgery occurred in the days or weeks preceding the disease. These infections resulted from native sources of these genera and not from consumption of probiotic products.

Two cases of *Lactobacillus* infection were linked with probiotic consumption (Borriello et al. 2003). Increasing consumption of probiotic lactobacilli and bifidobacteria has not led to an increase in such opportunistic infections in consumers (Salminen et al. 2002). Thus, the risk of infection by these genera is in the "negligible" range, taking into account that exposure to them is universal and persistent, not only through probiotic products but also as common colonizers of the human body (the digestive tract and oral and vaginal cavities). This lack of pathogenicity extends across all age groups (including preterm infants and pregnant women) (Lin et al. 2005; Saavedra et al. 2004).

Pharmaceutical or supplement forms of probiotics may be taken by consumers with less-than-optimal health status. These products also may encompass a broader range of microbes than typically used in foods, including members of the genera *Streptococcus*, *Enterococcus*, and *Escherichia*. Some strains or species of these genera are potentially pathogenic. In these instances, the accuracy of identification to the strain level is a critical step in the assessment of safety. Although an uncommon cause of infection in humans, a few cases of sepsis have been associated with administration of the probiotic *S. cerevisiae* (boulardii) (Munoz et al. 2005), sometimes caused by inadvertent contamination of intravenous lines.

Resistance to Antibiotics

Expression of resistance to antibiotics by candidate probiotic strains should be considered from the perspective of safety. But intrinsic resistance to some antibiotics is inherent to the physiology of certain probiotic strains because of their cell wall structure or other inherent physiological characteristics. The expression of resistance to an antibiotic is considered a risk factor if strains are suspected of harboring acquired, transferable antibiotic resistance genes, as suggested by expression of resistance to an antibiotic that exceeds the range determined to be normal for the species. In many instances, resistance to antibiotics is not transmissible, and the species also are sensitive to many other clinically used antibiotics. No particular safety concern, therefore, is associated with an intrinsic type of resistance.

Antibiotic resistance that is associated with transmissible genetic elements, however, should be avoided in bacteria intended for probiotic use because of the possibility of the resistance spreading to potentially harmful microbial residents (e.g., transmission of enterococcal resistance against the antibiotic, vancomycin). Vigilance regarding the detection of possible rare instances of infection due to probiotics should be maintained.

D-Lactate Acidosis

Another potential risk is the induction of D-lactate acidosis by D-lactic acid-producing probiotic bacteria. This is a very rare clinical condition that has never been linked to probiotic consumption. In fact, the occurrence of D-lactate acidosis has been recognized almost exclusively in patients with significantly decreased small intestinal absorptive capacity after intestinal bypass surgery or with short bowel syndrome (Mack 2004). In these instances, ingestion of carbohydrates may be followed by a massive load of sugars into the colonic lumen followed by production of D-lactate by commensal bacteria. Because humans metabolize D-lactate at a slower rate than the L-lactate isomer, increased absorption of D-lactate may lead to acidosis. Most subjects who have intestinal bypass operations or short bowel syndrome, however, do not develop D-lactic acidosis because the population of D-lactic acid-producing bacteria in the colonic microbiota of these subjects is not predominant. It is prudent, however, to recommend that D-lactate-producing probiotics be avoided by subjects with short bowel syndrome.

Product Considerations

Probiotic products are unique in that keeping the microbes alive must be a consideration through the stages of product concept, formulation, and the sales/distribution process. The typical issues surrounding product development also apply: products should be tasty (if in food form), convenient, and priced competitively. But additional considerations must be addressed: optimizing growth conditions for the probiotic, defining a product that can deliver the probiotic successfully in a viable and functional form to the active site in the body and through the end of shelf life in the product, and determining the role of the total product (including fermentation end products or other functional ingredients) on healthful properties. These considerations are not trivial, and unfortunately not all products marketed as "probiotic" suitably address them.

Several studies document examples of foods and supplements that either do not contain the amount of probiotic stipulated on the label or do not use the correct scientific nomenclature to name the microbes present. In addition, some

Table 3. Considerations for probiotic product development

Considerations	Comments
Probiotics should be described adequately and identified to the strain level.	Biochemical, morphological, physiological, and DNA-based techniques can contribute to the description of commercial probiotic strains. Total genomic DNA sequencing is becoming more common on fully characterized probiotic strains (Altermann et al. 2005).
Each probiotic strain should be able to be identified and enumerated from the product.	This can be a challenge because culture microbiology methods often are not available to differentiate among different species of the same genus. DNA-based approaches often can solve this problem.
Probiotic strains and products should be supported by a dossier substantiating efficacy.	A dossier should be developed that is composed at least in part of peer-reviewed publications documenting the ability of the probiotics to impact human health positively and supporting any claims made.
Product formulation should be evidence-based.	Decisions on product format (type of food or supplement), dose, and choice of strain(s) should be consistent with those used in clinical studies.
Viability of all probiotic strains in the product should be maintained above the target minimum level through the end of shelf life.	Viability (generally assessed as CFU/g) should be maintained at the level shown to deliver health effects. Although some decline in levels is common over time, initial formulations should assure that the levels do not drop below what is indicated on the label and what is known to be efficacious.
Product labeling should be done in a truthful and not misleading fashion	Product labels and any supplementary communications should provide clear, accurate information on the types and levels of probiotics; any documented health benefits; and the amount of product that must be consumed for an effect.
Probiotic strain(s) must be safe for their intended use.	(See main text)

products bear labels suggesting health effects that have not been documented. Some products (many yogurts fall into this category) do not make any claims of probiotic potency or efficacy but simply list the genus and species of additional live bacteria. The implication is that these bacteria are "good for you"; in fact, there may be little evidence that the products as formulated are efficacious.

Table 3 lists considerations for probiotic product development and expands on a document developed by a working group of the United Nations Food and Agriculture Organization (UNFAO/WHO 2002) that provides guidelines for probiotic products. This process involves choice of strain or combination of strains (not all strains of probiotics would be expected to function equally well in different roles), what amounts to use, what studies to conduct to document functionality, how to label the product, and how to communicate about the product. All these issues are interrelated and must start with an understanding of what health effect is envisioned for a product.

Regulatory Status of Probiotic Foods

Labeling of Probiotics

There is neither a legally recognized definition of, nor a standard of identity for, the term "probiotic" in the United States or worldwide. Products containing this label, therefore, currently are not obligated to meet any standards unique to probiotics. There is, however, a growing understanding of this term among consumers and healthcare professionals. It is unfortunate that products currently can be labeled as probiotics but be neither well defined nor substantiated with controlled human studies. Ideally, products labeled as probiotics would conform to the guidelines established by a working group of the FAO (UNFAO/WHO 2002). The requirement in the United States is that products be labeled in a truthful and not misleading fashion; this requirement applies to content as well as claims of functionality.

Many commercial products likely do not meet these criteria, as evidenced by several published surveys of commercial products. Most of these studies document the degree of label noncompliance with numbers or types of viable probiotic microbes recovered from commercial products (Drisko et al. 2005; Temmerman et al. 2003a, b; Yeung et al. 2002). Some of these failures are of more concern than others. Some instances of mislabeling the types of microbes present can be attributed to recent changes in scientific nomenclature; commercial product labels do not always reflect the most current, scientifically recognized nomenclature. For example, the species of *L. acidophilus* was subdivided into six different species (*L. acidophilus*, *L. gasseri, L. johnsonii, L. crispatus, L. gallinarum*, and *L. amylovoris*). Continued use of the *L. acidophilus* name instead of the newer name, although it is incorrect and should be corrected, is not of great significance. More important is that the microbes bear a consistent strain designation so that research can be tracked adequately for each strain even if the species name has been changed. One example of mislabeling at the genus level is with products claiming to contain "Lactobacillus sporogenes." This name was noted as a misclassification in 1939 (Sanders, Morelli, and Bush 2001). In fact, microbes using this name are most likely *Bacillus coagulans*. Its continued use commercially raises ethical, safety, and efficacy issues.

Studies also have documented failure of products to meet label claims with regard to numbers of viable microbes present in the product, thereby constituting illegally labeled products. Maintaining viability of microbes in commercial products through the end of shelf life can be a challenge. Viability

depends on many factors such as how the microbe is grown and stabilized and how it is handled (storage time, temperature, and exposure to moisture are important factors) once it is in the product. Although manufacturers' control over how retailers and consumers handle their products may be limited, storage and handling recommendations should be made; if those recommendations are met, products should meet label claims through the end of shelf life. But this is not the approach taken by all manufacturers and distributors of products currently in the marketplace.

Some product manufacturers make no claim to the quantities of microbes delivered. This practice is common in many food products, such as yogurt, that contain additional bacteria added for their health effects. For example, the label may indicate that the product "contains *L. acidophilus*," but no communication is provided on quantities or if the quantities added were chosen to match a documented health effect. In some instances, products might not indicate a quantity of probiotic on the label, but scrutiny of the product website might give some indication of levels delivered per serving. Some supplement products are labeled stating the quantity of viable probiotic per dose "at time of manufacture." This information is not very helpful for the consumer. Some supplements indicate dose to consume as a function of a gram or capsule amount but do not relate this to a viable cell count (CFU).

The most responsible approach to communicating the content of probiotic in products is to include on the label the genus, species, and strain designation for each probiotic in the product and the level of viable cells of each probiotic strain at the end of shelf life. Some manufacturers resist this approach, claiming it confuses consumers. Another acceptable choice is to provide this information through advertising or website communications. Additionally, the level needed to be consumed to achieve the documented health benefit should be disclosed.

Without any declaration of contents, however, consumers and healthcare professionals do not have adequate information to differentiate among products on the market. Documented failures of products to meet label claims with regard to numbers and types of viable microbes present in the product and how many must be consumed for a health benefit suggest that there is a problem in the probiotic industry with regard to accurate labeling. But not all such published studies have used methods that give accurate measures of bacterial content. Researchers unskilled in working with a particular species of probiotic or specific product formats may conclude erroneously that products do not contain indicated bacteria, whereas, in fact, their methods were inadequate.

A better approach for addressing this situation would be for companies to submit appropriate methods for assaying the contents of their products to a reliable third party for independent assessment. Making the results of such analyses available to consumers would assist in restoring confidence in products, a feeling that has eroded because of poor product formulation.

Safety Standards

Another important regulatory issue is safety. In the United States, there are different safety standards for different regulatory categories (foods, dietary supplements, or drugs) (Table 4). For supplements marketed before the October 15, 1994 passage of the Dietary Supplement Health and Education Act (DSHEA), it is the manufacturer's responsibility to ensure safety; no premarket approval is required. If a supplement product contains a "new dietary ingredient" or NDI (i.e., a dietary ingredient not sold in the United States in a dietary supplement before October 15, 1994 and not present in the food supply), then the manufacturer must notify the Food and Drug Administration (FDA) at least 75 days before marketing. If the FDA finds that the notice does not provide an adequate basis for believing that the ingredient is safe for its intended use, the Agency will warn the notifier that use of the ingredient in a dietary supplement may render it adulterated and liable to seizure.

The FDA has no authoritative list of dietary ingredients marketed before October 15, 1994, so the burden of proof rests with the manufacturer to determine if a new product is an NDI. If the probiotic was not sold in the United States as a dietary supplement before October 1994 and was not present in the food supply, then it is an NDI, and any dietary supplement containing it would be considered adulterated unless an NDI notification was filed with the FDA.

A "dietary ingredient" is defined by the DSHEA as one or any combination of the following substances: a vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by humans to supplement the diet by increasing the total dietary intake (e.g., enzymes or tissues from organs or glands); or a concentrate, metabolite, constituent, or extract (USFDA–CFSAN 2001). Although probiotics do not fall intuitively into any of these substance categories, the FDA has approved six probiotic bacteria as new dietary ingredients: *L. casei, L. reuteri, L. plantarum* (combined with fructooligosaccharide), *L. bulgaricus, L. paracasei*, and *B. infantis*. Other probiotic bacteria are sold in dietary supplements without having been the subjects of NDI reviews and may be regarded by the FDA as being "grandfathered" by use before October 1994. Note that FDA approval in this context is with regard to safety, not efficacy.

Generally Recognized as Safe

For conventional foods, it also is the manufacturer's responsibility to ensure safety; no premarket approval is required. All ingredients used in foods must be considered generally recognized as safe (GRAS), be a "Prior Sanctioned Substance" (sanctioned in a letter written by the FDA or the U.S. Department of Agriculture before 1958), or be a food additive approved by the FDA and used in compliance with an FDA food additive regulation. The GRAS substances are food substances judged by qualified subject experts as safe under the intended conditions of use. This determination may be based on data existing in the published scientific literature or through a long history of safe use. Although a limited list of GRAS substances is

Table 4. Differences among regulatory categories available for marketing probiotic products in the United States

	Regulatory Categories		
Actions	**Food**	**Supplement (must be ingested)**	**Drug**
Premarket approval by the FDA	Not required for GRAS microorganisms	Not required for microorganisms used before October 1994	Required
Disease claim (describes the effect of a drug on the diagnosis, treatment, mitigation, cure, or prevention of disease)	Not allowed	Not allowed	Allowed if approved by the FDA
Health claim (describes the effect of a dietary substance on the reduction of risk of disease by the currently healthy population)	Allowed if approved by the FDA (may be unqualified or qualified)	Allowed if approved by the FDA (may be unqualified or qualified)	Not used, although can use stronger prevention claims
Structure function claim (describes the effect of a dietary substance on the structure or function of the body)	Allowed if truthful and not misleading; effect must derive from the "nutritive value" of the food; no requirement for label disclaimer or FDA notification	Allowed if truthful and not misleading; commonly used; label must say "this statement has not been reviewed by the FDA"; must notify the FDA of intent to use this claim within 60 days of marketing the product	Not used
Safety standards[a]	Reasonable certainty of no harm under the intended conditions of use; GRAS status can be self-determined or submitted through GRAS notification process; must be safe for general population and all subgroups	No significant or unreasonable risk of illness or injury; target consumer group can be stipulated on the label	The FDA assesses safety and must determine that benefit outweighs risk
Product examples	Yogurt	Capsules	No probiotic products currently are regulated as drugs for human use in the United States

[a]These are not zero-risk standards.

published by the FDA, this list is not complete. Companies are allowed to conduct a "self-determination of GRAS status." The company may opt to notify the FDA of its findings, or it may keep the findings as an in-house document surrendered to the FDA only on their request. If a food substance is judged to be GRAS, either through the self-affirmation of GRAS status approach or through the FDA notification process, no premarket approval is required.

Only a few probiotic microbes are included on any official GRAS list. A common misconception is that "probiotics are GRAS." It should be kept in mind that the term GRAS applies to a food substance for a specified use. The term does not apply to drug uses and it does not apply to non-oral routes of administration. Manufacturers cannot assume that all probiotics are GRAS, even if they are composed of species of *Lactobacillus* or *Bifidobacterium*.

A responsible company should reflect seriously on all intended uses for all probiotic strains and strain blends it seeks to market and provide a considered rationale for GRAS status self-determination. Prior history of safe use of the species, negligible associations with infection or toxicity, and the absence of transferable antibiotic resistance genes from the specific strains being considered may comprise the bulk of the rationale. This endeavor becomes more complicated when dealing with other probiotic genera such as *Enterococcus, Streptococcus, Escherichia*, or *Bacillus*.

One difference in safety considerations for conventional foods compared with dietary supplements is that conventional foods are marketed to the general population. It is allowable to label dietary supplements for use in particular subpopulations (e.g., adults, or children over the age of 3 years), but this practice is not allowed on conventional foods. Also, foods may be consumed for an entire lifetime, so cumulative effects need to be considered. Further, dose amounts may be recommended for dietary supplements, but food ingredients must be safe even for people who may consume far more than average amounts.

The European Food Safety Authority (EFSA) recently proposed the "qualified presumption of safety" (QPS) approach as an operating tool for safety assessment of microorganisms in food and feed (EFSASC 2004). This system is based on the taxonomic identification of the candidate microorganisms and the body of knowledge concerning the particular species of microorganism, including history of use, scientific literature and databases, clinical aspects, ecology, and industrial applications. The result of this effort will be a list of QPS microbes that will be considered safe for use in foods.

Regulating Claims of Efficacy

The U.S. regulatory status of claims that can be made for conventional foods and dietary supplements is summarized in an online document from the U.S. Food and Drug Administration–Center for Food Safety and Applied Nutrition (USFDA–CFSAN 2003). The responsibility for ensuring the validity of these claims rests with the manufacturer, the FDA, or—with regard to advertising—the Federal Trade Commission (FTC); the validity is determined on a case-by-case basis. Although probiotics most commonly are considered as components of functional foods or dietary supplements, they also may be suitable for treatment or mitigation of disease and, as such, could be marketed as drugs. No such products, however, have entered the U.S. market for human use. Two important differences among these categories are how statements about health or disease can be made on the label and what can be said in advertising of these different product categories (Sanders et al. 2005). The FTC has long used a "reasonable consumer" standard for determining whether messages are truthful and not misleading. The FDA adopted the reasonable consumer standard in 2002, superseding the previous standard of "the ignorant, the unthinking, and the credulous." The FDA has provided guidance on what constitutes substantiation for claims of efficacy on dietary supplements (USFDA–CFSAN 2004).

Since the enactment of the DSHEA in 1994, a commonly expressed sentiment is that dietary supplements are "unregulated." This point is illustrated by comments made in a television news report aired May 8, 2006 on probiotics in which a University of California–Berkeley professor was quoted as saying, "Right now, it's the Wild West. There is no quality control. There is no standardization. There is no proof of efficacy, and there is no proof of safety" (Mulvihill 2006).

Although it is true that dietary supplements are not regulated in the same manner as drugs—with preapproval for efficacy and safety and mandatory analyses of every batch produced to assure potency and purity—there are regulations issued and enforced by the FDA and the FTC to prevent the use of untested or unsafe ingredients, to require the use of good manufacturing practices, and to compel truth in labeling and marketing. Unfortunately, enforcement rigor on the part of these agencies is limited. An examination of warning letters reveals that the focus of enforcement efforts is on the mislabeling of supplements as drugs through use of unapproved drug claims and removal of unsafe products; no warning letters were issued to a food or supplement manufacturer for claims of efficacy that were unsubstantiated. This circumstance reflects the FDA's priorities: first, safety, including hazards presented by leading consumers to forego needed pharmacological intervention through false claims; second, filth; and—a distant third—economic deception.

The lack of FDA enforcement, however, does not diminish the responsibility of companies to substantiate any claims of efficacy. Even structure/function claims such as "improves microbial balance" or "enhances natural immune function" must be based on evidence derived from human studies on the strain or strains and levels used. Strain- and dose-specificity of probiotic function must be presumed unless demonstrated otherwise, and simply providing a source of live cultures is not sufficient to support such claims.

Future Developments

The field of probiotics is growing rapidly with concomitant developments in the research, commercial, and medical sectors. The complete genomic sequences are known for several important probiotic bacteria, and functional genomics findings will be instrumental in identifying many features responsible for probiotic functionality. According to Klaenhammer and colleagues (2005), "This information is providing an important platform for understanding core mechanisms that control and regulate bacterial growth, survival, signaling, and…underlying probiotic activities within complex microbial and host ecosystems." Genetic approaches also will enable design of genetically modified probiotic strains with specific therapeutic capabilities such as delivery of anti-inflammatory cytokines, vaccine epitopes, or antipathogenic molecules. Well-designed and properly controlled human and mechanistic studies will advance the essential understanding of active principles, mechanisms of action, and degree of effects that can be realized by specific consumer groups.

In the commercial realm, the success of probiotics in Europe and Asia likely will be realized in the United States. The number and types of products will increase in the food, supplement, and pharmaceutical categories. As competition intensifies in the marketplace, companies providing responsibly formulated and promoted products will prevail. Lastly, as this field advances, look for new types of probiotic strains with benefits not yet explored that may surpass the value of those currently in commercial use.

Recommendations

1. **Establish a standard of identity for the term "probiotic" based on the FAO definition** (UNFAO/WHO 2001). Adoption of a standard of identity for use of the term would assure that the word retains some meaning in the marketplace and that only well-defined products with documented efficacy could use this term on labels.
2. **Regulate probiotics based on their intended use, but expand regulatory conceptualization of health benefit claims.** In the United States, probiotics have been used as food ingredients, medical foods, and supplements and are in development as drugs. Different regulatory requirements exist for each of these categories and are imposed from the research and development stages through to marketing of a product. The FDA should be encouraged to explore how it might expand its approach to appropriate targets for health benefit claims, and related research to substantiate those claims, for foods. Foods (including those containing probiotics) often are useful to provide support to improve tolerance or otherwise complement drug therapy. Probi-

otic foods also may be of benefit to help at-risk individuals avoid disease, including acute conditions such as bacterial vaginosis, necrotizing enterocolitis, and antibiotic-associated diarrhea. Studies that validate such effects, and communication of these benefits **for foods**, should be facilitated, not suppressed, by regulatory agencies.

3. **Adopt the use of third party verification of label claims.** This practice would go far toward increasing consumer confidence in probiotic products and would decrease the oversight needed by governmental agencies. Verification could address both content and efficacy claims, resulting in the ability of consumers and healthcare professionals to better differentiate among commercial products.
4. **Use probiotics selectively in clinical conditions.** It is common to field questions from some people who are desperately hoping that probiotics will solve a certain critical health concern. The use of probiotics for new medical applications is not recommended in clinical conditions in which there is no evidence of benefit that is based on positive results in well-designed and well-performed clinical trials. The use of probiotics in a new clinical setting or condition should be accepted only after consultation and approval by the ethics committee of a clinical research institution.
5. **Consider multiple factors when evaluating probiotics.** When considering the body of evidence supporting probiotics and their impact on human health, it should be kept in mind that human studies are necessary for confirming benefits. Research evaluating probiotics should follow standard best clinical practice guidelines. But there are additional factors to consider such as the contribution of the delivery vehicle, including bioactive ingredients intrinsic to the carrier or resulting from growth or metabolism of the probiotic during product preparation; the need to specify and verify the dose of each component of the probiotic being tested; and the need to identify the genus, species, and strain of each strain tested. Editors of journals publishing papers on probiotics should be aware of the importance of these disclosures.
6. **Focus research on the important role of human native microbiota in health.** The role that native, colonizing microbes play (such as improving vaccine efficiency, improving outcomes of drug therapy, improving tolerance to drug therapy, or improving resistance to infections—especially ones of global magnitude) is an important public health concern. Influencing these microbial populations or activities through the use of probiotics, and the causal relationship between these changes and validated health biomarkers or clinical effects, is a recommended line of research.
7. **Use a science-based assessment of the benefits and risks of genetically engineered probiotic microbes.** Although not addressed in this review, the use of genetically engineered probiotic microbes to treat or prevent disease shows potential. Although some people advocate an outright ban on such use, a more appropriate approach is a science-based assessment of safety and environmental exposure risk compared with the benefits that recombinant organisms might provide.
8. **Provide better information to consumers.** Consumers are asking basic questions about probiotics such as: What are they? How many do I need to consume? What kinds are best? How often do I need to consume them? Are they safe? What effects can I expect? Unbiased, straightforward answers to these types of questions need to be developed and disseminated to the general public.

Appendix 1. How Probiotics Work

The beneficial effects of probiotics likely result from several complex, interacting mechanisms that will differ for different strains and sites of action. These mechanisms may include competition for binding sites to the intestinal wall, competition for essential nutrients, production of antimicrobial substances, stimulation of *mucin* production, stabilization of the intestinal barrier, improvement of gut transit, metabolism of nutrients to volatile fatty acids, and immunomodulation (immune stimulation and immunoregulation). Some of these mechanisms have been demonstrated only through laboratory experiments or animal models and are not substantiated in humans.

Contrary to popular belief, probiotics generally have not been shown to have a large or consistent impact on populations of intestinal microbes, and any effect they do exert is generally transient. This is likely because the native microbiota are a well-adapted, naturally stable association of microbes that effectively resist change (see review issued by the French Food Safety Agency [AFSSA 2005]). Probiotics, however, have been shown to impact the biochemistry of the intestinal environment, and administration of probiotics during periods of disruption of normal microbial balance (such as during antibiotic therapy) seems to provide opportunity for probiotics to influence health.

The recognition during the past decade of the limitations of scientists' knowledge of the composition of the intestinal populations of microbes has resulted in a shift of focus from probiotic-induced changes in populations of microbes to more readily measurable end points. Perhaps more important than documenting an impact on populations of intestinal microbes is the ability to demonstrate that probiotics can promote the recovery of disturbed microbial populations (Engelbrektson et al. 2006), improve the character of the intestinal environment, or impact validated indicators of human health.

Regulation of the Immune System

Of paramount importance to the function of many probiotic bacteria is their ability to impact the immune system. This is perhaps the most broadly studied mechanism of probiotic impact on human health and provides a key means by which probiotics may mediate effects all through the body of the host. Different probiotics are able to stimulate, as well as regulate, several aspects of natural and acquired immune responses. This interaction has broad-reaching significance to human health and could impact infectious disease, response to vaccines, cancer, allergic disease, autoimmune disorders, and inflammatory diseases. Animal studies show that different strains of probiotics can have different effects on the immune system. Effects also are dependent on dose and on immune status of the host. Different probiotics can stimulate immune responses or down-regulate inflammatory responses. A thorough review of this research is beyond the scope of this paper, and the reader is referred to recent reviews for more details (Cummings et al. 2004; Erickson and Hubbard 2000; Gill and Cross 2001; Gill and Guarner 2004; Guarner 2006; Madsen 2006).

The major cellular effectors of innate immunity include epithelial cells, phagocytic cells (monocytes, macrophages, and neutrophils), and natural-killer cells (NK-cells). Probiotics have been found to modulate the functions of all these cells (Gill and Guarner 2004). *Acquired immunity* comprises antibody- and cell-mediated responses and is characterized by its specificity and memory. Consumption of specific probiotics has been shown to enhance antibody responses to natural infections and to immunizations. For example, Kaila and colleagues (1992) found significantly higher levels of specific antibody responses in children with rotavirus if the children consumed *L. rhamnosus* GG-fermented milk. *Cytokines* are the largest and most diverse group of immune response mediators. Initiation, maintenance, and resolution of both innate and acquired immune responses are regulated by cytokines. The ability of probiotics to induce cytokine production by a range of immune cells may explain how they are able to influence both innate and acquired immune responses.

Studies on this topic frequently are conducted in laboratory or animal models. Although extremely valuable to understanding the nature of probiotic effects on the various components of the immune system, these types of studies cannot provide the essential insights into the extent to which probiotics impact health or disease states. Most human studies measure changes in markers of general immune function. The fate of these markers, however, fails to illustrate fully the impact of probiotics on human health in both healthy and diseased subjects. There are no agreed on, definitive markers for the influence of probiotics on the immune system.

Understanding this limitation, several animal and human studies have provided evidence that specific probiotic strains are able to influence several aspects of natural and acquired immune responses. Immunological sensing of probiotic bacteria in the gut is performed by specialized cells overlying the *Peyer's patches* and by the cells lining the intestinal walls. *Dendritic cells* distributed throughout the region under the epithelial cells also have been shown to have the ability to sample antigens directly from the intestinal contents, providing an important link between *luminal contents* and systemic immune responses. In the end, however, controlled human studies documenting that probiotics decrease the incidence, duration, or severity of symptoms of infection or cancer are more meaningful.

Delivery of Proteins

Probiotic bacteria are capable of delivering enzymes or other functional proteins. As discussed previously, perhaps the best example of this is the microbe-mediated delivery of the enzyme that turns lactose into the more readily digested glucose and galactose in people unable to digest lactose fully. Some probiotic-derived enzymes may help to digest food that enters the small and large intestines (the end products of which are harmless or even beneficial). Braat and colleagues (2006) successfully used a genetically engineered *Lactococcus* to deliver the cytokine IL-10, which can decrease the inflammatory response, to the intestinal tracts of Crohn's patients. The treatment induced remission in five of the ten patients treated in the study, and three others showed clinical signs of improvement.

Producing Antimicrobial Substances

Several probiotic bacteria have been shown to produce a range of antimicrobial substances including organic acids (lactic acid and acetic acid), hydrogen peroxide, carbon dioxide, and diacetyl, as well as *bacteriocins* and bacteriocin-like substances (Mishra and Lambert 1996; Ouwehand et al. 1999). Both lactic and acetic acids inhibit microbes by decreasing the pH of the intestinal contents, which retards every aspect of bacterial metabolism (Mishra and Lambert 1996). Hydrogen peroxide inhibits the growth of both *Gram-positive* and *Gram-negative* bacteria (Hollang, Knapp, and Shoesmith 1987; Mishra and Lambert 1996). Diacetyl exerts its growth-inhibitory effect by interfering with *arginine* utilization by reacting with arginine-binding proteins (Jay 1986).

Bacteriocins are defined as proteins or protein complexes of high molecular weight produced by certain bacteria that kill bacteria, usually closely related to the strain producing the bacteriocin (Klaenhammer 1988). Probiotic bacteria have been shown to produce two types of antibacterial substances: low molecular weight antimicrobial substances (e.g., reuterin, produced by *L. reuteri*) and bacteriocins (Ouwehand 1998). Whether all or some of these substances are produced by these bacteria once they are inside the host is not known. Recently, however, Corr and colleagues (2007) documented that an anti-*Listeria* activity observed in animals fed a bacteriocin-producing strain of *Lactobacillus salivarius* was lost in mutants no longer able to produce the bacteriocin. This is the first definite

proof that pathogen inhibition results directly from bacteriocin production by the probiotic. Pathogens including *E. coli, Salmonella, Campylobacter, Shigella, Vibrio, Clostridium, Candida albicans*, human immunodeficiency virus, and other viruses have been inhibited in laboratory tests or animal studies by bacteriocin-producing probiotic strains (O'Sullivan and Kullen 1998). But proof that the bacteriocin—and not some other cell function—mediates observed antipathogenic effects in vivo still is needed for most examples of bacteriocins.

Stimulation of Mucin Production

A gel-like mucus layer composed of complex proteins bound with sugar molecules (mucins) covers the intestinal surfaces. The mucus layer shields the gut surface from direct contact with the contents passing through the intestine and acts as a barrier against invasion by pathogenic organisms and toxins (Sanderson and Walker 1994; Yolken et al. 1994). Mucus can protect against enteric pathogens by serving as a physical barrier, by housing antibodies that can bind potentially harmful antigens, and by releasing mucins into the intestinal tract thereby removing bound pathogens from the intestinal cells (Dai et al. 2000).

It is well documented that some members of the natural microbiota are able to degrade intestinal mucin, whereas others are able to stimulate mucus secretion (Kohler, McCormick, and Walker 2003). Mack and colleagues (1999) showed that certain probiotics are able to influence the regulation of mucin production. Growth of probiotics (*L. rhamnosus* GG and *L. plantarum* 299V) with colon cells in laboratory studies resulted in stimulation of mucin production. Furthermore, these strains were effective at inhibiting enterohemorrhagic *E. coli* attachment to mucus-producing intestinal cells, but not to non-mucus-secreting cells, thus suggesting a protective role of mucin. The ability of probiotic strains to impact mucus production is an elegant example of communication of the bacteria with the host and may provide a mechanism by which probiotics can decrease the likelihood of infection by pathogens.

Stabilization of Gut Mucosal Barrier

The native microbes colonizing human bodies play an important role in preserving the integrity and function of the barrier between the contents of the intestine and the inside of bodies. This integrity can be compromised by pathogenic bacteria, toxins, inflammation, or stress, leading to intestinal permeability and unwanted transfer of bacterial (including resident microbiota) and dietary antigens across the gut wall. This transfer leads to activation of immune responses that can result in inflammatory and autoimmune disorders. Increased gut permeability is a characteristic feature of food allergies and immunoinflammatory gut diseases.

Recent studies have shown that intake of specific probiotics is effective in preventing and repairing damage to the lining of the intestine. Some animal studies have demonstrated reduced gut permeability associated with cow's milk feeding (Isolauri et al. 1993) and inflammatory bowel disease (Madsen et al. 2001). Probiotics may mediate these effects in several ways: by inhibiting damage to intestinal cell junctions (Luyer et al. 2005; Montalto et al. 2004); improving cell growth and survival (Otte and Podolsky 2004); inducing mucin secretion (Mack et al. 2003); promoting tissue repair (Yamaguchi, Yan, and Polk 2003); decreasing bacterial adhesion (Sherman et al. 2005); and secreting repair factors and nutrients (e.g., short-chain fatty acids, polyamines, nitric acid, and stimulating production of secretory immunoglobulin A [IgA]) (Viljanen et al. 2005). The ability of probiotics to produce factors that directly strengthen intestinal barrier integrity and protect against pathogenic bacteria also has been reported (Madsen et al. 2001).

Glossary

Acquired immunity. Immunity resulting from previous exposure to an infectious agent or antigen (e.g., vaccine).

Alimentary canal. The system, or organs, from the mouth to the anus that takes in food, digests it to extract energy and nutrients, and expels the remaining waste.

Arginine. A naturally occurring amino acid.

Bacteriocin. A high molecular weight protein or protein complex produced by bacteria that kills bacteria closely related to the producer bacteria.

Colonization. The state of microbes becoming established and growing in a particular environmental niche. In the intestinal tract, this state may be achieved through direct attachment of the microbe to intestinal cells (adherence), or may be due to association with the mucus layer lining the intestinal wall.

Colonization resistance. Limiting action of the normal flora on colonization of the bowel by potentially pathogenic microorganisms.

Colony forming units (CFU). The measure of the count of bacteria when assayed on solid growth media, or an agar plate. The bacterial suspension is diluted serially so that a small amount spread on the surface of an agar plate leaves between 30 and 300 bacteria. The plate is incubated until each bacterial cell or chain of cells forms a colony visible with the naked eye. After figuring in the dilution factor, this assay results in a count (CFU) per ml or g of test product.

Crohn's disease. A chronic, episodic, inflammatory condition of the gastrointestinal tract (mouth to anus) characterized by inflammation and lesions.

Culture methodologies. Methods to enumerate microorganisms based on the process of dilution to extinction and subsequent plating on agar plates containing nutrients necessary for the growth and incubation under the appropriate conditions of the particular microbe being assayed.

Cytokines. Regulatory proteins and peptides released by many different cell types for the purpose of signaling other cells. Cytokines are particularly important for the initiation and

regulation of both innate and adaptive immune responses. Cytokines also are involved in several developmental processes during embryogenesis.

Dairy products with lactose. Lactose is a carbohydrate (composed of one glucose molecule bound to one galactose molecule) unique to mammalian milk. Its biological role is to provide a carbohydrate source for nursing mammals. It is present at about 5% by weight in cow's milk. Lactose is naturally present in many dairy foods such as milk, yogurt, ice cream, buttermilk, cottage cheese, and other soft cheeses. Aged cheeses usually do not contain significant lactose because much of the lactose comes off with the whey during manufacture and the remaining lactose is fermented by cultures during the aging process. Lactose in yogurt is only partially fermented so that yogurt has approximately the same lactose content as milk.

Dendritic cells. Immune cells that process and present antigens on their surface to other cells of the immune system. They are present in small numbers in tissues that are in contact with the external environment, including skin, the inner lining of the nose, lungs, stomach, and intestines. They also can be found at an immature state in the blood. Once activated, dendritic cells migrate to the lymphoid tissues where they interact with T cells and B cells to initiate and shape the immune response.

Double-blind, clinical trial. Double-blind describes a clinical trial in which neither the subject nor the investigator (researcher) knows what treatment/supplement a subject is receiving.

Enteric coated. Enteric coating is a barrier applied to oral supplements/drugs that controls the location in the digestive system where the coated substance is released. This process can protect probiotics from exposure to harmful gastric acid and pancreatic secretions and improve survival through intestinal transit.

Genus. The taxonomic level of division between the Family and the Species.

Gram-positive, **Gram-negative**. Named by the Danish bacteriologist who developed the test, the Gram reaction is a color-binding assay (purple – Gram positive; pink – Gram negative) that reveals fundamental cell surface characteristics of bacteria. All lactic cultures (e.g., *Lactobacillus, Bifidobacterium, Streptococcus*) and most probiotic genera are Gram-positive.

Ileum. The most distal region of the small intestine.

Innate (nonspecific) immune system. The first line of defense against infections, innate immunity does not demonstrate immunological memory. The innate immune system gives rise to the acute inflammatory response and has limited specificity for microbes. Phagocytes dominate this response.

Irritable bowel syndrome. An intestinal disorder characterized most commonly by cramping, abdominal pain, bloating, constipation, and diarrhea that cannot be attributed to any other known disease.

Jejunum. The middle section of the small intestine extending from the duodenum to the ileum. The jejunum is the longest section of the small intestine.

Locally. In this context, locally refers to effects that occur at the site of exposure to probiotics. For example, the production of organic acids in the colon by probiotic bacteria can inhibit pathogens in the colon.

Luminal contents. The contents of the lumen, or the interior of the intestinal tract.

Meta-analysis. The process of using statistical methods to combine the results of different studies on the same topic to determine the strength of overall conclusions that can be made on the effect of the treatment.

Microbiota. Term that refers to the collective body of microorganisms inhabiting an ecosystem. Sometime called "microflora," this latter term is not taxonomically correct because bacteria are not plants or flora.

Mucin. A family of large, heavily glycosylated proteins, membrane-bound or secreted on mucosal surfaces.

Mucosal immune system. The immune system associated with mucous membranes of the respiratory, gastrointestinal, and urogenital tracts.

Oligofructose. Also known as fructooligosaccharide, the prebiotic, oligofructose, is a short chain of fructose molecules that reaches the colon and serves as a substrate for growth of colonic microbes.

Pancreatic secretions. Secretions from the pancreas (pancreatic juice) containing digestive enzymes, including trypsinogen, chymotrypsinogen, elastase, carboxypeptidase, pancreatic lipase, and amylase. The alkaline nature of pancreatic juice neutralizes gastric acid, improving digestive enzyme effectiveness.

Peyer's patch cell. Peyer's patches, named after the 17th-century Swiss anatomist Hans Conrad Peyer, are organized lymphoid tissue (secondary lymphoid organs) found in the wall of the small intestine.

Pouchitis. Inflammation of a surgically constructed pouch after removal of the large bowel, often in patients with ulcerative colitis.

Prebiotics. Nondigestible substances that, when consumed, provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria.

Probiotics. Live microorganisms that confer a health benefit on the host when administered in adequate amounts.

Species. A taxonomic unit that for bacteria refers to the degree of relatedness among individuals assigned to the species, and the degree of difference among individuals of other species. In general, members of the same bacterial species are 95% similar to each other based on their chromosomal DNA.

Strain. A designation for a specific individual member of a species. Strains are genetically homogeneous. Many different strains can be part of the same species.

Synbiotic. A product containing both a

probiotic and a prebiotic. Evidence for synergy or specificity of the probiotic for the prebiotic is not essential.

Systemically. In this context, systemically refers to effects that occur distant from the site of exposure to probiotics (extra-intestinally). These effects can be mediated by activated, circulating immune cells.

Transit time. The time it takes for food and resulting digesta to move through the alimentary canal.

Ulcerative colitis. A form of inflammatory bowel disease that affects the large intestine or colon and is characterized by inflamation and ulcers in the colon.

Literature Cited

Agence Francaise de Sécurité Sanitaire des Aliments/French Food Safety Agency (AFSSA). 2005. *Effects of Probiotics and Prebiotics on Flora and Immunity in Adults*, http://www.usprobiotics.org/docs/AFFSA%20probiotic%20prebiotic%20flora%20immunity%2005.pdf (4 October 2006)

Altermann, E., W. M. Russell, M. A. Azcarate-Peril, R. Barrangou, B. L. Buck, O. McAuliffe, N. Souther, A. Dobson, T. Duong, M. Callanan, S. Lick, A. Hamrick, R. Cano, and T. R. Klaenhammer. 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proc Natl Acad Sci USA* 102(11):3906–3912.

Anderson, A. D., C. E. McNaught, P. K. Jain, and J. MacFie. 2004. Randomised clinical trial of synbiotic therapy in elective surgical patients. *Gut* 53:241–245.

Anukam, K. C., E. Osazuwa, G. I. Osemene, F. Ehigiagbe, A. W. Bruce, and G. Reid. 2006a. Clinical study comparing probiotic lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes and Infection* 8:1–5.

Anukam, K., E. Osazuwa, I. Ahonkhai, M. Ngwu, G. Osemene, A. W. Bruce, and G. Reid. 2006b. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: Randomized, double-blind, placebo controlled trial. *Microbes and Infection* 8:1450–1454.

Bausserman, M. and S. Michail. 2005. The use of *Lactobacillus* GG in irritable bowel syndrome in children: A double-blind randomized control trial. *J Pediatr* 147:197–201.

Bin-Nun, A., R. Bromike, M. Wilschanski, M. Kaplan, B. Rudensky, M. Caplan, and C. Hammerman. 2005. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr* 147:192–196.

Borriello, S. P., W. P. Hammes, W. Holzapfel, P. Marteau, J. Schrezenmeir, M. Vaara, and V. Valtonen. 2003. Safety of probiotics that contain lactobacilli or bifidobacteria. *Clin Infect Dis* 36:775–780.

Bousvaros, A., S. Guandalini, R. N. Baldassano, C. Botelho, J. Evans, G. D. Ferry, B. Goldin, L. Hartigan, S. Kugathasan, J. Levy, K. F. Murray, M. Oliva-Hemker, J. R. Rosh, V. Tolia, A. Zholudev, J. A. Vanderhoof, and P. L. Hibberd. 2005. A randomized, double-blind trial of *Lactobacillus* GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis* 11:833–839.

Braat, H., P. Rottiers, D. W. Hommes, N. Huyghebaert, E. Remaut, J. Remon, S. J. H. van Deventer, S. Neirynck, M. P. Peppelenbosch, and L. Steidler. 2006. A Phase I trial with transgenic bacteria expressing Interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 4(6):754–759.

Brouwer, M. L., S. A. Wolt-Plompen, A. E. Dubois, S. van der Heide, D. F. Jansen, M. A. Hoijer, H. F. Kauffman, and E. J. Duiverman. 2006. No effects of probiotics on atopic dermatitis in infancy: A randomized placebo-controlled trial. *Clin Exp Allergy* 36(7):899–906.

Bu, L. N., M. H. Chang, Y. H. Ni, H. L. Chen, and C. C. Cheng. 2007. *Lactobacillus casei rhamnosus* Lcr35 in children with chronic constipation. *Pediatr Int* 49(4):485–490.

Corr, S. C., Y. Li, C. U. Riedel, P. W. O'Toole, C. Hill, and C. G. Gahan. 2007. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci USA* 104(18):7617–7621.

Cummings, J. H., J. M. Antoine, F. Azpiroz, R. Bourdet-Sicard, P. Brandtzaeg, P. C. Calder, G. R. Gibson, F. Guarner, E. Isolauri, D. Pannemans, C. Shortt, S. Tuijtelaars, and B. Watzl. 2004. PASSCLAIM—Gut health and immunity. *Eur J Nutr* 43(Suppl. 2): II/118–173.

Dai, D., N. Nanthkumar, D. S. Newburg, and W. A. Walker. 2000. Role of oligosaccharides and glycoconjugates in intestinal host defense. *J Pediatr Gastroenterol Nutr* 30:S23–S33.

Daniel, C, S. Poiret, D. Goudercourt, V. Dennin, G. Leyer, and B. Pot. 2006. Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Appl Environ Microbiol* 72(9):5799–5805.

de Vrese, M., A. Stegelmann, B. Richter, S. Fenselau, C. Laue, and J. Schrezenmeir. 2001. Probiotics—compensation for lactase insufficiency. *Am J Clin Nutr* 73(Suppl):421S–429S.

de Vrese, M., P. Winkler, P. Rautenberg, T. Harder, C. Noah, C. Laue, S. Ott, J. Hampe, S. Schreiber, K. Heller, and J. Schrezenmeir. 2005. Effect of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5 on common cold episodes: A double blind, randomized, controlled trial. *Clin Nutr* 24(4):481–491.

Drisko, J., B. Bischoff, C. Giles, M. Adelson, R. V. Rao, and R. McCallum. 2005. Evaluation of five probiotic products for label claims by DNA extraction and polymerase chain reaction analysis. *Dig Dis Sci* 50(6):1113–1117.

Eckburg, P. B., E. M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson, and D. A. Relman. 2005. Diversity of the human intestinal microbial flora. *Science* 308(5728):1635–1638.

Engelbrektson, A. L., J. R. Korzenik, M. E. Sanders, B. G. Clement, G. Leyer, T. R. Klaenhammer, and C. L. Kitts. 2006. Analysis of treatment effects on the microbial ecology of the human intestine. *FEMS Microbiol Ecol* 57(2):239–250.

Erickson, K. L. and N. E. Hubbard. 2000. Probiotic immunomodulation in health and disease. *J Nutr* 130:403S–409S

Eriksson, K., B. Carlsson, U. Forsum, and P. G. Larsson. 2005. A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. *Acta Derm Venereol* 85(1):42–46.

European Food Safety Authority Scientific Colloquium (EFSASC). 2004. Microorganisms in food and feed: Qualified presumption of safety—QPS, http://www.efsa.europa.eu/en/science/colloquium_series/no2_qps.html (4 October 2006)

Galdeano, C. M., A. de Moreno de LeBlanc, G. Vinderola, M. E. Bonet, and G. Perdigon. 2007. Proposed model: Mechanisms of immunomodulation induced by probiotic bacteria. *Clin Vaccine Immunol* 14(5):485–492.

Gasser, F. 1994. Safety of lactic-acid bacteria and their occurrence in human clinical infections. *Bulletin de L'Institut Pasteur* 92:45–67.

Gibson, G. R. 2005. The rise and rise of probiotics. *Biologist* 52:95–98.

Gill, H. S. and M. L. Cross. 2001. Probiotics and immune function. Pp. 251–272. In P. Calder, H. S. Gill, and C. Field (eds.). *Nutrition and Immunity*. CABI, New York.

Gill, H. S. and F. Guarner. 2004. Probiotics and human health: A clinical perspective. *Postgrad Med J* 80:516–526.

Gionchetti, P., F. Rizzello, A. Venturi, P. Brigidi, D. Matteuzzi, G. Bazzocchi, G. Poggioli, M. Miglioli, and M. Campieri. 2000. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: A double-blind, placebo-controlled trial. *Gastroenterology* 119:305–309.

Gionchetti, P., F. Rizzello, U. Helwig, A. Venturi, K. M. Lammers, P. Brigidi, B. Vitali, G. Poggioli, M. Miglioli, and M. Campieri. 2003. Prophylaxis of pouchitis onset with probiotic therapy: A double-blind, placebo-controlled trial. *Gastroenterology* 124:1202–1209.

Guarner, F. 2006. Enteric flora in health and disease. *Digestion* 73(Suppl 1):5–12.

Hickson, M., A. L. D'Souza, N. Muthu, T. R. Rogers, S. Want, C. Rajkumar, and C. J. Bulpitt. 2007. Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: Randomised double blind placebo controlled trial. *BMJ* 335(7610):80.

Hlivak, P., J. Odraska, M. Ferncik, L. Ebringer, E. Jahnova, and Z. Mikes. 2005. One-year application of probiotic strain *Enterococcus faecium* M-74 decreases serum cholesterol levels. *Bratisl Lek Listy* 106(2):67–72.

Hollang, K. T., J. S. Knapp, and J. G. Shoesmith. 1987. *Anaerobic Bacteria*. 1st ed. Blackie and Son, Ltd., London.

Holzapfel, W. H., P. Haberer, J. Snel, U. Schillinger, H. J. Jos, and H. I. Veld. 1998. Overview of gut flora and probiotics. *Inter J Food Microbiol* 41:85–101.

Ishikawa, H., I. Akedo, T. Otani, T. Suzuki, T. Nakamura, I. Takeyama, S. Ishiguro, E. Miyaoka, T. Sobue, and T. Kakizoe. 2005. Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. *Int J Cancer* 116:762–767.

Isolauri, E., H. Majamaa, T. Arvola, I. Rantala, E. Virtanen, and H. Arvilommi. 1993. *Lactobacillus case*i strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 105(6):1643–1650.

Jauhiainen, T., H. Vapaatalo, T. Poussa, S. Kyronpalo, M. Rasmussen, and R. Korpela. 2005. *Lactobacillus helveticus* fermented milk lowers blood pressure in hypertensive subjects in 24-h ambulatory blood pressure measurement. *Am J Hypertens* 18(12 Pt 1):1600–1605.

Jay, J. M. 1986. *Modern Food Microbiology*. 1986. 3rd ed. Van Nostrand Reinhold, New York.

Kaila, M., E. Isolauri, E. Soppi , E. Virtanen, S. Laine, and H. Arvilommi. 1992. Enhancement of the circulating antibody secreting cell response in human diarrhoea by a human *Lactobacillus* strain. *Pediatric Res* 32:141–144.

Kajander, K., K. Hatakka, T. Poussa, M. Farkkila, and R. A. Korpela. 2005. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: A controlled 6-month intervention. *Aliment Pharmacol Ther* 22:387–394.

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

Kalliomaki, M., S. Salminen, T. Poussa, H. Arvilommi, and E. Isolauri. 2003. Probiotic and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 361:1869–1871.

Kato, K., S. Mizuno, Y. Umesaki, Y. Ishii, M. Sugitani, A. Imaoka, M. Otsuka, O. Hasunuma, R. Kurihara, A. Iwasaki, and Y. Arakawa. 2004. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther* 20: 1133–1141.

Kim, H. J., M. Camilleri, S. McKinzie, M. B. Lempke, D. D. Burton, G. M. Thomford, and A. R. Zinsmeister. 2003. A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* 17: 895–904.

Kim, H. J., M. I. Vazquez Roque, M. Camilleri, D. Stephens, D. D. Burton, K. Baxter, G. Thomforde, and A. R. Zinsmeister. 2005. A randomized controlled trial of a probiotic combination VSL# 3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil* 17:687–696.

Kirjavainen, P. V., S. J. Salminen, and E. Isolauri. 2003. Probiotic bacteria in the management of atopic disease: Underscoring the importance of viability. *J Pediatr Gastroenterol Nutr* 36:223–227.

Klaenhammer, T. R. 1988. Bacteriocins of lactic acid bacteria. *Biochimie* 70:337–379.

Klaenhammer, T. R., R. Barrangou, B. L. Buck, M. A. Azcarate-Peril, and E. Altermann. 2005. Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS Microbiol Rev* 29(3):393–409.

Kohler, H., B. A. McCormick, and W. A. Walker. 2003. Bacterial-enterocyte crosstalk: Cellular mechanisms in health and disease. *J Pediat Gastroenterol Nutr* 36:175–185.

Kolars, J. C., M. D. Levitt, M. Aouji, and D. A. Savaiano. 1984. Yogurt—An autodigesting source of lactose. *New Engl J Med* 310:1–3.

Kruis, W., E. Schutz, P. Fric, B. Fixa, G. Judmaier, and M. Stolte. 1997. Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 11:853–858.

Kruis, W., P. Fric, J. Pokrotnieks, M. Lukas, B. Fixa, M. Kascak, M. A. Kamm, J. Weismueller, C. Beglinger, M. Stolte, C. Wolff, and J. Schulze. 2004. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gut* 53:1617–1623.

Kukkonen, K., E. Savilahti, T. Haahtela, K. Juntunen-Backman, R. Korpela, T. Poussa, T. Tuure, and M. Kuitunen M. 2007. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: A randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 119(1):192–198.

Lin, H. C., B. H. Su, A. C. Chen, T. W. Lin, C. H. Tsai, T. F. Yeh, and W. Oh. 2005. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 115:1–4.

Lionetti, E., V. L. Miniello, S. P. Castellaneta, A. M. Magista, A. de Canio, G. Maurogiovanni, E. Ierardi, L. Cavallo, and R. Francavilla. 2006. *Lactobacillus reuteri* therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: A randomized placebo controlled trial. *Aliment Pharmacol Ther* 24(10):1461–1468.

Luyer, M. D., W. A. Buurman, M. Hadfoune, G. Speelmans, J. Knol, J. A. Jacobs, C. H. C. Dejong, A. J. M. Vriesema, and J. W. M. Greve. 2005. Strain-specific effects of probiotics on gut barrier integrity following hemorrhagic shock. *Infection and Immunity* 73:3686–3692.

Macfarlane, G. T., S. Macfarlane, and G. R. Gibson. 1998. Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colonic microbiota. *Microbial Ecol* 35:180–187.

Mack, D. R. 2004. D(–)-lactic acid-producing probiotics, D(–)-lactic acidosis and infants. *Can J Gastroenterol* 18:671–675.

Mack, D. R., S. Michail, S. Wei, L. McDougall, and M. A. Hollingsworth. 1999. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing mucin gene expression. *Amer J Physiol* 276:G941–G950.

Mack, D. R., S. Ahrne, L. Hyde, S. Wei, and M. A. Hollingsworth. 2003. Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. *Gut* 52(6):827–833.

Madsen, K. 2006. Probiotics and the immune response. *J Clin Gastroenterol* 40:232–234.

Madsen, K., A. Cornish, P. Soper, C. McKaigney, H. Jijon, C. Yachimec, J. Doyle, L. Jewell, and C. De Simone. 2001. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 121:580–591.

Marteau, P., P. Seksik, and R. Jian. 2002. Probiotics and intestinal health effects: A clinical perspective. *Br J Nutr* 88(Suppl 1):S51–S57.

Marteau, P., B. Flourie, P. Pochart, C. Chastang, J-F. Desjeux, and J-C. Rambaud. 1990. Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: An *in vivo* study on lactase deficient humans. *Br J Nutr* 64:71–79.

Marteau, P., E. Cuillerier, S. Meance, M. F. Gerhardt, A. Myara, M. Bouvier, C. Bouley, F. Tondu, G. Bommelaer, and J. C. Grimaud. 2002. *Bifidobacterium animalis* strain DN-173 010 shortens the colonic transit time in healthy women: A double-blind, randomized, controlled study. *Aliment Pharmacol Ther* 16:587–593.

McCartney, A. L. 2002. Application of molecular biological methods for studying probiotics and the gut flora. *Br J Nutr* 88:S29–S37.

McFarland, L. V. 2006. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. *Am J Gastroenterol* 101(4):812–822.

Mimura, T., R. Rizzello, U. Helwig, G. Poggioli, S. Schreiber, I. C. Talbot, R. J. Nicholls, P. Gionchetti, M. Campieri, and M. A. Kamm. 2004. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 53:108–114.

Mishra, C. and J. Lambert. 1996. Production of anti-microbial substances by probiotics. *Asia Pacific J Clin Nutr* 5:20–24.

Montalto, M., N. Maggiano, R. Ricci, V. Curigliano, L. Santoro, F. Di Nicuolo, F. M. Vecchio, A. Gasbarrini, and G. Gasbarrini. 2004. *Lactobacillus acidophilus* protects tight junctions from aspirin damage in HT-29 cells. *Digestion* 69:225–228.

Montrose, D. C. and M. H. Floch. 2005. Probiotics used in human studies. *J Clin Gastroenterol* 39(6):469–484.

Mulvihill, K. 2006. *'Good Germs' Good for You*, http://cbs5.com/health/local_story_129011601.html (2 June 2006)

Munoz, P., E. Bouza, M. Cuenca-Estrella, J. M. Eiros, M. J. Perez, M. Sanchez-Somolinos, C. Rincon, J. Hortal, and T. Pelaez. 2005. *Saccharomyces cerevisiae fungemia*: An emerging infectious disease. *Clin Infect Dis* 40(11):1625–1634.

Myllyluoma, E., L. Veijola, T. Ahlroos, S. Tynkkynen, E. Kankuri, H. Vapaatalo, H. Rautelin, and R. Korpela. 2005. Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy—a placebo-controlled, double-blind randomized pilot study. *Aliment Pharmacol Ther* 21:1263–1272.

Nase, L., K. Hatakka, E. Savilahti, M. Saxelin, A. Ponka, T. Poussa, R. Korpela, and J. H. Meurman. 2001. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res* 35(6):412–420.

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

O'Hara, A. M. and F. Shanahan. 2007. Mechanisms of action of probiotics in intestinal diseases. *Sci World J* 7:31–46.

Olah, A., T. Belagyi, A. Issekutz, M. E. Gamal, and S. Bengmark. 2002. Randomized clinical trial of specific *Lactobacillus* and fibre supplement to early enteral nutrition in patients with acute pancreatitis. *Br J Surg* 89:1103–1107.

O'Mahony, L., J. McCarthy, P. Kelly, G. Hurley, F. Luo, K. Chen, G. C. O'Sullivan, B. Kiely, J. K. Collins, F. Shanahan, and E. M. Quigley. 2005. *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology* 128:541–551.

O'Sullivan, D. J. and M. J. Kullen. 1998. Tracking of probiotic bifidobacteria in the intestine. *Intl Dairy J* 8:513–525.

Otte, J. M. and D. K. Podolsky. 2004. Functional modulation of enterocytes by Gram-positive and Gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 286: G613–G626.

Ouwehand, A. C. 1998. Antimicrobial components from lactic acid bacteria. Pp. 139–159. In S. Saliminen and A. von Wright (eds.). *Lactic Acid Bacteria: Microbiology and Functional Aspects*. 2nd ed. Marcel Dekker, New York.

Ouwehand, A. C., P. V. Kirjavainen, C. Shortt, and S. Salminen. 1999. Probiotics: Mechanisms and established effects. *Intl Dairy J* 9:43–52.

Perdigon, G. and R. Fuller (eds.). 2003. *Gut Flora, Nutrition, Immunity and Health.* Blackwell Publishing, Oxford.

Picard, C., J. Fioramonti, A. Francois, T. Robinson, F. Neant, and C. Matuchansky. 2005. Review article: Bifidobacteria as probiotic agents—physiological effects and clinical benefits. *Aliment Pharmacol Ther* 22(6):495–512.

Prantera, C., M. L. Scribano, G. Falasco, A. Andreoli, and C. Luzi. 2002. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: A randomised controlled trial with *Lactobacillus* GG. *Gut* 51:405–409.

Rayes, N., D. Seehofer, T. Theruvath, R. A. Schiller, J. M. Langrehr, S. Jonas, S. Bengmark, and P. Neuhaus. 2005. Supply of pre- and probiotics reduces bacterial infection rates after liver transplantation—A randomized, double-blind trial. *Am J Transplant* 5:125–130.

Reid, G. and A. Bocking. 2003. The potential for probiotics to prevent bacterial vaginosis and preterm labor. *Am J Obstet Gynecol* 189(4):1202–1208.

Reid, G., D. Beuerman, C. Heinemann, and A. W. Bruce. 2001. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. *FEMS Immunol Med Microbiol* 32(1):37–41.

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

Rosenfeldt, V., E. Benfeldt, S. D. Nielsen, K. F. Michaelsen, D. L. Jeppesen, N. H. Valerius, and A. Paerregaard. 2003. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol* 111:389–395.

Rumney, C. J. and I. R. Rowland. 1992. *In vivo* and *in vitro* models of the human colonic flora. *Crit Rev Food Sci Nutr* 31(4):299–331.

Saavedra, J. M. 2007. Use of probiotics in pediatrics: Rationale, mechanisms of action, and practical aspects. *Nutr Clin Pract* 22(3):351–365.

Saavedra, J. M., N. A. Bauman, I. Oung, J. A. Perman, and R. H. Yolken. 1994. Feeding of *Bifido-bacterium bifidum* and *Streptococcus termophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 334:1046–1049.

Saavedra, J. M., A. Abi-Hanna, N. Moore, and R. H. Yolken. 2004. Long-term consumption of infant formulas containing live probiotic bacteria: Tolerance and safety. *Am J Clin Nutr* 79:261–267.

Salminen, M. K., S. Tynkkynen, H. Rautelin, M. Saxelin, M. Vaara, P. Ruutu, S. Sarna, V. Valtonen, and A. Jarvinen. 2002. *Lactobacillus* bacteremia during a rapid increase in probiotic use of *Lactobacillus rhamnosus* GG in Finland. *Clin Infect Dis* 35:1155–1160.

Salminen, S., C. Bouley, M-C. Boutron-Ruault, J. H. Cummings, A. Franck, G. R. Gibson, E. Isolauri, M. C. Moreau, M. B. Roberfroid, and I. R. Rowland. 1998. Functional food science and gastrointestinal physiology and function. *Br J Nutr* 80:S147–S171.

Sanders, M. E., L. Morelli, and S. Bush. 2001. "*Lactobacillus sporogenes*" is not a *Lactobacillus* probiotic. *ASM News* 67(8):385–386.

Sanders, M. E., T. Tompkins, J. T. Heimbach, and S. Kolida. 2005. Weight of evidence needed to substantiate a health effect for probiotics and prebiotics: Regulatory considerations in Canada, E.U., and U.S. *Eur J Nutr* 44(5):303–310.

Sanderson, I. R. and W. A. Walker. 1994. Mucosal barrier. Pp. 41–51. In P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. McGhee, and J. Bienenstock (eds). *Handbook of Mucosal Immunology*. Academic Press, Inc., London.

Saran, S., S. Gopalan, and T. P. Krishna. 2002. Use of fermented foods to combat stunting and failure to thrive. *Nutrition* 18:393–396.

Savino, F., E. Pelle, E. Palumeri, R. Oggero, and R. Miniero. 2007. *Lactobacillus reuteri* (American Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: A prospective randomized study. *Pediatrics* 119(1):e124–130.

Sazawal, S., G. Hiremath, U. Dhingra, P. Malik, S. Deb, and R. E. Black. 2006. Efficacy of probiotics in prevention of acute diarrhoea: A meta-analysis of masked, randomised, placebo-controlled trials. *Lancet Infect Dis* 6(6):374–382.

Sheil, B., F. Shanahan, and L. O'Mahony. 2007. Probiotic effects on inflammatory bowel disease. *J Nutr* 137(3 Suppl. 2):819S–824S.

Sherman, P. M., K. C. Johnson-Henry, H. P. Yeung, P. S. C. Ngo, J. Goulet, and T. A. Tompkins. 2005. Probiotics reduce enterohemmorrhagic *Escherichia coli* 0157:H7- and Enteropathogenic *E. coli* 0127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. *Infection and Immunity* 73:5183–5188.

Sheu, B. S., H. C. Cheng, A. W. Kao, S. T. Wang, Y. J. Yang, H. B. Yang, and J. J. Wu. 2006. Pretreatment with *Lactobacillus*- and *Bifidobacterium*-containing yogurt can improve the efficacy of quadruple therapy in eradicating residual *Helicobacter pylori* infection after failed triple therapy. *Am J Clin Nutr* 83(4):864–869.

Shortt, C. and J. O'Brien (eds.). 2004. *Handbook of Functional Dairy Products*. CRC Press, Boca Raton, Florida.

Sykora, J., K. Valeckova, J. Amlerova, K. Siala, P. Dedek, S. Watkins, J. Varvarovska, F. Stozicky, P. Pazdiora, and J. Schwarz. 2005. Effects of a specially designed fermented milk product containing probiotic *Lactobacillus casei* DN-114 001 and the eradication of *H. pylori* in children: A prospective randomized double-blind study. *J Clin Gastroenterol* 39:692–698.

Szajewska, H., M. Kotowska, J. Z. Mrukowicz, M. Armanska, and W. Mikolajczyk. 2001. Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants. *J Pediatr* 138:361–365.

Szajewska, H. M. Ruszczynski, and A. Radzikowski. 2006. Probiotics in the prevention of antibiotic-associated diarrhea in children: A meta-analysis of randomized controlled trials. *J Pediatr* 149(3):367–372.

Tannock, G. W. (ed.). 2005. *Probiotics and Prebiotics: Scientific Aspects*. Caister Academic Press, Wymondham, U. K.

Temmerman, R., I. Scheirlinck, G. Huys, and J. Swings. 2003a. Culture-independent analysis of probiotic products by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 69(1):220–226.

Temmerman, R., B. Pot, G. Huys, and J. Swings. 2003b. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *Int J Food Microbiol* 81(1):1–10.

Tubelius, P., V. Stan, and A. Zachrisson. 2005. Increasing work-place healthiness with the probiotic *Lactobacillus reuteri*: A randomised, double-blind placebo-controlled study. *Environ Health* 4:25.

Turchet, P., M. Laurenzano, S. Auboiron, and J. M. Antoine. 2003. Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114001 on winter infections in free-living elderly subjects: A randomised, controlled pilot study. *J Nutr Health Aging* 7(2):75–77.

U.S. Food and Drug Administration–Center for Food Safety and Applied Nutrition (USFDA–CFSAN). 2001. *Overview of Dietary Supplements*, http://www.dfsan.fda.gov/~dms/ds-oview.html#what (20 October 2006)

U.S. Food and Drug Administration–Center for Food Safety and Applied Nutrition (USFDA–CFSAN). 2003. *Claims that Can Be Made for Conventional Foods and Dietary Supplements*, http://www.cfsan.fda.gov/~dms/hclaims.html (12 February 2007)

U.S. Food and Drug Administration–Center for Food Safety and Applied Nutrition (USFDA–CFSAN). 2004. *Substantiation for Dietary Supplement Claims Made under Section 403(r) (6) of the Federal Food, Drug, and Cosmetic Act*, http://www.cfsan.fda.gov/~dms/dsclmgui.html (12 February 2007)

United Nations. Food and Agriculture Organization of the United Nations/World Health Organization (UNFAO/WHO). 2001. *Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*, http://ftp.fao.org/es/esn/food/probio_report_en.pdf (4 October 2006)

United Nations. Food and Agriculture Organization of the United Nations/World Health Organization (UNFAO/WHO). 2002. *Guidelines for the Evaluation of Probiotics in Food*, http://ftp.fao.org/es/esn/food/wgreport2.pdf (4 October 2006)

Van Loo, J., Y. Clune, M. Bennett, and J. K. Collins. 2005. The SYNCAN project: Goals, set-up, first results and settings of the human intervention study. *Br J Nutr* 93(Suppl 1):S91–S98.

Viljanen, M., E. Savilahti, T. Haahtela, K. Juntunen-Backman, R. Korpela, T. Poussa, T. Tuure, and M. Kuitunen. 2005. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: A double-blind placebo-controlled trial. *Allergy* 60:494–500.

Weizman, Z., G. Asli, and A. Alsheikh. 2005. Effect of a probiotic infant formula on infections in child care centers: Comparison of two probiotic agents. *Pediatrics* 115(1):5–9.

Wendakoon, C. N., A. B. Thomson, and L. Ozimek. 2002. Lack of therapeutic effect of a specially designed yogurt for the eradication of *Helicobacter pylori* infection. *Digestion* 65:16–20.

Weston, S., A. Halbert, P. Richmond, and S. L. Prescott. 2005. Effects of probiotics on atopic dermatitis: A randomised controlled trial. *Arch Dis Child* 90:892–897.

Willis, C. L., G. R. Gibson, J. Holt, S. Atherton, and C. Allison. 1999. Negative correlation between oral malodour and numbers and activities of sulphate-reducing bacteria in the human mouth. *Arch Oral Biol* 44:665–670.

Yamaguchi, D. J., F. Yan, and D. B. Polk. 2003. Probiotic *Lactobacillus rhamnosus* GG stimulates proliferation during intestinal epithelial cell wound repair. *J Pediatr Gastroenterol Nutr* 37:395.

Yeung, P. S., M. E. Sanders, C. L. Kitts, R. Cano, and P. S. Tong. 2002. Species-specific identification of commercial probiotic strains. *J Dairy Sci* 85(5):1039–1051.

Yolken, R. H., C. Ojeh, I. A. Khatri, U. Sajjan, and J. F. Forstner. 1994. Intestinal mucins inhibit rotavirus replication in an oligosaccharide-dependent manner. *J Infect Dis* 169:1002–1006.

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