

Making Sense of Probiotics

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INTRODUCTION

Consider for a moment that the human gut contains 10 times more bacteria than all the human cells in the entire body.¹ The intestinal tract is home to approximately 100,000,000,000,000 (100 trillion) microorganisms.² This enormous biomass consists of over 400 known diverse bacterial species that generate intense metabolic activity and are of key importance for human health.¹ In addition to promoting normal gastrointestinal functions and providing protection from infection, the intestinal microflora also exerts important effects on systemic metabolism and immune function. While the exact roles of many of these organisms are not yet clearly understood, basic scientific and clinical research is beginning to characterize the diverse functions of normal intestinal microbiota. Probiotics are either member species of the essential intestinal microflora or they are transient species affecting benefit as they pass through the gastrointestinal tract. This article summarizes the present knowledge of probiotics, reviews their uses and applications supporting health, and answers commonly asked questions about probiotics.

PROBIOTICS - Definition and Species

In the early 1900s, the Nobel laureate Metchnikoff reported favorable health effects and improved longevity from consuming fermented milk products.³ He suggested that ingestion of live lactic acid bacteria may improve the balance of the gastrointestinal microflora. In 1965, Lilly and Stillwell introduced the term “probiotics” for growth-promoting factors produced by microorganisms.⁴ Fuller popularized the word “probiotic” in 1989, describing probiotics as live microbial feed supplements, which benefit the host by improving intestinal microbial balance.⁵ In 2001, an International Life Sciences Institute Europe consensus document proposed a simple and now widely accepted definition of probiotics as “viable microbial food supplements which beneficially influence the health of humans.”⁶ Probiotics consist of lactic acid producing bacteria (LAB), non-lactic acid producing bacterial species, and non-pathogenic yeast.

LACTIC ACID PRODUCING PROBIOTICS

Lactobacillus

The genus *Lactobacillus* normally predominates in the small intestine. *Lactobacillus* species are facultative anaerobes although some species, such as *Lactobacillus plantarum*, can respire oxygen turning it into hydrogen peroxide. Of the more than 100 *Lactobacillus* species, the following are commonly used probiotics:

<i>L. acidophilus</i>	<i>L. fermentum</i>	<i>L. paracasei</i>
<i>L. brevis</i>	<i>L. gasseri</i>	<i>L. plantarum</i>
<i>L. bulgaricus</i>	<i>L. helveticus</i>	<i>L. reuteri</i>
<i>L. casei</i>	<i>L. jensenii</i>	<i>L. rhamnosus</i>
<i>L. crispatus</i>	<i>L. johnsonii</i>	<i>L. salivarius</i>

Bifidobacterium

Bifidobacterium is another well-documented genus of lactic acid producing bacteria. Bifidobacteria are strictly anaerobic and normally

vie for predominance in the large intestine. The following 8 of the more than 30 *Bifidobacterium* species are frequently used as probiotics:

<i>B. adolescentis</i>	<i>B. breve</i>	<i>B. longum</i>
<i>B. animalis</i>	<i>B. infantis</i>	<i>B. thermophilum</i>
<i>B. bifidum</i>	<i>B. lactis</i>	

Streptococcus

Streptococcus species are not typically associated with health benefits and often highly pathogenic. However, one facultative anaerobic species, *Streptococcus thermophilus*, is known to promote health. It is one of the two primary species found in yogurt cultures, the other being *L. bulgaricus*.

Enterococcus

Found in a number of probiotic products, the facultative anaerobe *Enterococcus faecium* has a variety of beneficial characteristics. However, *E. faecium* has evolved from a relatively nonpathogenic commensal bacteria to the third most common cause of hospital acquired infections and now accounts for over 10% of enterococcal clinical isolates.^{7,8} It has developed extensive resistance to antibiotics.

NON-LACTIC ACID-PRODUCING PROBIOTICS

Bacillus

Bacillus species are ubiquitous facultative or obligate aerobic, spore-producing organisms found in the soil and water.⁹ Spores of a number of *Bacillus* species are used as probiotics and are often referred to as soil-based probiotics. A number of species including *Bacillus subtilis*, *B. coagulans*, *B. licheniformis*, and *B. cereus* have shown benefit. However, *Bacillus* species have well documented toxicities that include the potential production of enterotoxins. *Bacillus* probiotic products have been plagued by problems with mislabeling and associated with gastroenteritis and diarrhea.⁹

Propionibacterium

Propionibacterium species are Gram-positive, nonsporing, pleomorphic rods first described in 1906.¹⁰ Usually anaerobic, some strains tolerate very small amounts of air (microaerophilic). Their primary fermentation products are propionic acid, acetic acid, and carbon dioxide. *Propionibacterium* species are commonly found on the skin. Propionobacteria stimulate the growth of bifidobacteria, reduce pathogenic fecal *Staphylococcus* and *Enterobacteriaceae* populations, decrease the fecal concentration of carcinogenic enzymes, and favorably modulate the immune system. Select *Propionibacterium* species may have good potential as probiotics.

YEAST PROBIOTICS

Saccharomyces

The yeast genus *Saccharomyces* contains 7 or 10 species of which only *S. boulardii* is used as a probiotic.¹¹ Unaffected by gastric acid and bile, *S. boulardii* proliferates along the entire gastrointestinal tract. It has been used alone and in combination with other probiotics to successfully manage a variety of gastrointestinal disorders especially diarrhea and *Clostridium difficile*-associated disease.^{11,12}

PROBIOTICS - Major Species and Characteristics

Lactobacillus

Lactobacillus species are facultative anaerobic, Gram-positive, non-spore forming rods or elongated ovals (cocci/bacilli). They are characterized as homofermentative, meaning they primarily produce lactic acid as a fermentation end-product, or heterofermentative, meaning lactic acid, carbon dioxide, ethanol, and acetic acid are the principal fermentation end-products.¹³ Since the advent of gene typing and hybridization technologies, *Lactobacillus* classification has evolved rapidly and there are presently over 100 accepted species.¹⁴ *Lactobacilli* possess many important features that make them valuable probiotics. These include production of enzymes to digest and metabolize proteins and carbohydrates, synthesis of B vitamins and vitamin K, breakdown of bile salts, enhancement of innate and acquired immunity, and inhibition of proinflammatory mediators. *Lactobacillus* species exhibit antimicrobial activities against an array of pathogens including *Pseudomonas*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Candida*, and *Helicobacter pylori*.

L. acidophilus is undoubtedly the best known probiotic. For decades a variety of lactobacilli were misclassified as *L. acidophilus*. Only in recent years have these organisms been recognized as distinct species with distinguishing features and unique potential health benefits. One confusing result of new, refined methods of microbial classification is that many of the healthful effects long attributed to “*L. acidophilus*” are now recognized to belong to other *Lactobacillus* species. One such species, *L. rhamnosus*, is now appreciated as highly beneficial. It was not until 1989 that *L. rhamnosus* was recognized as a separate species and it was not viewed as beneficial for many years. Evolving reclassification of “*L. acidophilus*” species was largely responsible for the results of Hughes’ 1990 study of probiotics that found almost none of the “*L. acidophilus*” probiotics tested contained *L. acidophilus*. The most commonly identified species was *L. rhamnosus*.

L. rhamnosus strains are probably the most extensively studied probiotics. Many studies have repeatedly found that *L. rhamnosus* GG can treat and prevent rotavirus diarrhea, prevent antibiotic-associated diarrhea, and treat diarrhea caused by *Clostridium difficile*.^{6,12,17} *L. rhamnosus* has significant immunomodulatory properties. The effects of *L. rhamnosus* GG were examined in infants with allergies to cow’s milk and atopic dermatitis and associated with significant improvements compared to placebo.¹⁸ The probiotic reduced several markers of intestinal inflammation in the infants possibly due to improved intestinal barrier function leading to decreases in antigen translocation. Two more recent studies have also demonstrated the benefits of *L. rhamnosus* GG in preventing and treating atopic dermatitis and eczema in infants.^{19,20}

L. acidophilus was once thought to be indigenous to the human gastrointestinal tract and that consuming *L. acidophilus* restocked the intestines with normal microflora. *L. acidophilus* is now known not to be indigenous to the bowel, but species previously classified as *L. acidophilus*, such as *L. gasseri*, *L. crispatus*, and *L. johnsonii*, are indigenous.²¹⁻²³ Most *Lactobacillus* probiotics are not indigenous to the human gastrointestinal tract, but colonize the intestines when regularly consumed. Vegetarians and people ingesting traditional plant-based diets have high colonization rates of certain lactobacilli such as *L. plantarum*, *L. rhamnosus*, and *L. acidophilus*. Colonization rates with these important microorganisms are low in individuals consuming a standard highly processed Western. It is now clear that probiotics must be regularly consumed to restore and maintain the normal intestinal balance of essential microorganisms.

Bifidobacterium

Bifidobacteria were identified at the end of the 19th century as irregular Y-shaped bacteria and termed *Bacillus bifidus*.²⁴ For most of the 20th century they were classified as members of the genus *Lactobacillus* because they produced lactic acid. Numerous studies detailing a unique physiology and nutritive requirements led to the creation of distinct genus called *Bifidobacterium* presently composed of over 30 species. Bifidobacteria are strictly anaerobic, non-spore forming rods. They are among the more common LAB in the human intestinal tract competing with *Bacteroides* species for predominance in the colon. They constitute 95% of the gut bacterial population in healthy, breast-fed infants.²⁵ *Bifidobacterium* populations tend to remain stable in the adult human intestine, but may decline with age; the decline may even contribute to aging.²⁶ Their numbers are devastated by antibiotics and other environmental toxins. The presence of *Bifidobacterium* within the intestinal tract is associated with numerous health benefits.

The list of health benefits for probiotic *Bifidobacterium* is extensive. Nutritionally, they all metabolize lactose, generate the L(+) form of lactic acid, synthesize certain vitamins, ferment indigestible carbohydrates, and produce beneficial short-chain fatty acids. *B. bifidum*, *B. breve*, and *B. lactis* all exhibit protective effects against acute diarrhea. *B. longum* and *B. bifidum* have been shown to reduce the incidence and duration of antibiotic-associated diarrhea as well as traveler’s diarrhea. They inhibit pathogens principally by the production of organic acids and hydrogen peroxide and through stimulation of the host immune system. *Bifidobacterium* species have been found to relieve constipation, alleviate inflammatory bowel disease, and reduce serum cholesterol levels. In animal models, *B. longum* and *B. breve* have been shown to prevent DNA damage which suggests probiotics may prevent or delay the onset of certain cancers.²⁵

Saccharomyces boulardii

S. boulardii, formally known as *S. cerevisiae* variant *boulardii* Hansen CBS 5926, is a non-colonizing, lactic acid producing yeast.¹¹ It has been widely used worldwide as a beneficial probiotic. Clinical trials have shown that *S. boulardii* prevents or treats many intestinal maladies including antibiotic-associated diarrhea, recurrent *Clostridium difficile*-associated disorders, acute diarrhea, traveler’s diarrhea, and diarrhea in tube-fed patients. In adults, *S. boulardii* has been successfully used to treat AIDS-related diarrhea and to prevent relapses of Crohn’s disease and ulcerative colitis. *S. boulardii* exerts direct protective effects against the enteric pathogens *Vibrio cholerae* and *E. coli*. It is known to exert several beneficial effects on the host gastrointestinal tract through diverse mechanisms of action. *In vivo*, *S. boulardii* secretes proteases and other substances that break down bacterial enterotoxins and inhibit their binding to intestinal receptors. *S. boulardii* stimulates host immune defenses, reduces intestinal secretions, inhibits enterotoxin-induced inflammatory responses and enhances production of intestinal trophic factors such as brush border membrane enzymes and nutrient transporters.

Streptococcus thermophilus and Lactobacillus bulgaricus

These two species of LAB are the primary cultures used in yogurt production.²⁷ While both species are transient and do not colonize the intestinal tract, they have significant health benefits. They metabolize lactose, improving lactose intolerance. *In vitro* studies have shown that these two species have potent antimicrobial activities against *Pseudomonas*, *E. coli*, *Staph. aureus*, *Salmonella*, and *Shigella*. In some cases, this antimicrobial activity was compared to that of *L. acidophilus* and found to be stronger. *L. bulgaricus* has also shown *in vitro* activity against *H. pylori*. These two yogurt culture bacteria have been used for

millennia to promote health and longevity and will, without doubt, have a continuing vital role as probiotic supplements.

Enterococcus faecium

E. faecium, formerly called *Streptococcus faecium*, is a ubiquitous organism found in a variety of foods as well as in soil and on plants. It colonizes the skin, intestinal tract, and genitals in humans. *E. faecium* is a hearty species capable of surviving higher temperatures and lower pHs than other probiotics. Several studies have suggested *E. faecium* effectively prevents and resolves antibiotic-associated diarrhea and it has been used to treat acute gastroenteritis. *In vivo* and *in vitro* studies have demonstrated an inhibiting effect against several pathogenic organisms including *Staph. aureus*, *E. coli*, *Salmonella*, *Clostridium*, and *Listeria*.²⁸ No longer a nonpathogenic commensal bacterium, *E. faecium* has increasingly become capable of causing severe, often life-threatening infections.⁷ The acquisition of antibiotic resistance by enterococcal species is a growing clinical problem. At present about one-half of *E. faecium* clinical isolates are resistant to the critically important antibiotic vancomycin.²⁹ The risks of *E. faecium* use as a probiotic are rapidly becoming greater than the potential benefits.

PROBIOTICS - Health Benefits

Probiotic organisms have been shown to be effective in a variety of both gastrointestinal and extra-intestinal conditions beyond modulating intestinal microflora and replenishing and maintaining normal gut commensal bacterial equipoise.

Diarrhea

The benefits of probiotics have been clearly documented in four types of diarrhea: antibiotic-associated diarrhea, *Clostridium difficile*-associated diarrhea (CDAD), rotavirus diarrhea, and infectious diarrhea.^{1,30-32} Antibiotic-associated diarrhea and CDAD are the best documented conditions that may be prevented or treated with probiotics.^{12,33} A meta-analysis of 25 randomized, controlled trials involving 2,810 patients concluded that probiotics significantly reduced the relative risk of antibiotic-associated diarrhea by 57%.¹² Three types of probiotics were found to be most beneficial: *S. boulardii*, *L. rhamnosus* GG, and multispecies probiotic combinations. A meta-analysis of 23 randomized, controlled trials conducted by the United Kingdom West Midlands Health Technology Assessment Group found that probiotics significantly reduced the relative risk of CDAD by 46%.³⁴ *S. boulardii* has been consistently found to reduce the risk of new and recurrent cases of CDAD. *S. boulardii* has been found to be particularly beneficial for adults who have suffered more than one bout of recurrent CDAD.^{35,36} Rotavirus-induced diarrhea, a common problem in hospitalized children, has been shown to be prevented by *L. rhamnosus*, *L. casei*, *S. thermophilus*, and *B. bifidum*.⁶ *L. rhamnosus*, *L. reuteri*, *L. casei*, and *S. boulardii* have been found to either effectively prevent or treat community-acquired infectious diarrhea in infants and children.¹

Vaginal dysbiosis

Dysbiosis refers to the disruption of the normal microbial ecosystems in body tissues that may lead to clinical symptoms and disease. As in the intestinal tract, the normal vaginal microflora can be disrupted and undesirable microorganisms can proliferate especially during and following courses of antibiotics. Historically, preparations of LAB, introduced either as yogurt soaked tampons or douches or encapsulated probiotic suppositories, have been used to check the growth of pathogens with favorable clinical results. Unfortunately, studies on the efficacy of probiotics for vaginal infections often have mixed results, probably due to differences in study design, selection of proper probiotic strains, probiotic viability, and other factors. However, clinicians and patients alike have typically found that vaginal

infections commonly recur when drugs alone are prescribed, while better outcomes are achieved when probiotics are applied vaginally concomitantly with medications.^{32,37} Direct vaginal application may not be necessary as studies show that orally administered probiotics can reduce the incidence of recurrent yeast vaginitis, bacterial vaginosis, and urinary tract infections.³⁸

Antagonism to pathogens

The use of probiotics for both intestinal and vaginal disorders hinges on the ability of specific strains to antagonize the growth of disease-causing organisms. In the intestinal tract, a delicate balance constantly needs to be maintained between beneficial and pathogenic organisms. A variety of factors can shift the intestinal microflora balance in favor of pathogens. These factors include antibiotics, immunosuppressants, stress, aging, poor diet, excessive alcohol intake, environmental pollutants, and infections. Many studies have confirmed that probiotics promote a more favorable balance of intestinal microflora by reducing populations of harmful microorganisms. Probiotics accomplish this task primarily by producing substances toxic to pathogenic organisms such as lactic acid, acetic acid, formic acid, hydrogen peroxide, and bacteriocins.¹⁵ Probiotic bacteria also compete with pathogens for nutrients and living space in the gut. Bifidobacteria are capable of absorbing large quantities of ferrous iron, depriving pathogens of iron and inhibiting their growth. Clinically, the re-establishment of a favorable bowel microflora balance in the gut may manifest short-term as a resolution of diarrhea or other gastrointestinal symptoms. Long-term, a re-established healthy balance may reduce the risk of a variety of chronic degenerative or immunologically-mediated diseases.^{17,39}

Immune function enhancement

The intestines are the primary immune organ in the body. The bowel-associated immune system contains the largest mass of lymphoid tissue in the human body, a vitally important component of total host immunologic capacity.⁴⁰ Bowel mucosa and lymphoid tissue are closely linked immunologically with gastrointestinal microflora. Substantial evidence associates probiotic bacteria with modulation of host-mediated immune responses. Probiotic bacteria boost both innate and acquired immune responses. These include increases in circulating lymphocytes, stimulation of phagocytosis and antigen-specific antibody secretion, and increased production of interferon- γ and other cytokines. Immunologic enhancement properties are best documented for *L. casei*, *L. rhamnosus*, *L. plantarum*, *L. bulgaricus*, *L. acidophilus*, *B. bifidum*, and *B. breve*. While these species are almost certainly not the only probiotics that modulate immune function, they should definitely be part of any therapeutic probiotic regimen to support the immune system.⁴⁰⁻⁴²

Digestive support

Most lactic acid probiotic bacteria are capable of metabolizing a variety of carbohydrates, including lactose. LAB metabolism of lactose is what enables many lactose-intolerant people to consume yogurt, but not other dairy products. LAB ferment carbohydrates into other short- and medium-chain organic acids in addition to lactic acid. Some LAB species also secrete proteolytic and lipolytic enzymes that facilitate digestion of proteins and fats. People who produce inadequate amounts of stomach acid and cannot activate the proteolytic enzyme pepsin and individuals with pancreatic insufficiency deficient in pancreatic proteases and lipases all benefit from dietary supplementation with probiotics. Enhanced protein digestion often benefits people with allergies due to increased gut permeability defects by reducing the ability of large proteins to cross the intestinal barrier, enter the bloodstream, and trigger immune responses.^{42,43}

Short-chain fatty acid production

Probiotics especially the bifidobacteria, are able to break down and metabolize non-digestible carbohydrates such as fiber. The major by-products of this process are short-chain fatty acids (SCFA) such as lactate, acetate, propionate, and butyrate. SCFA lower intestinal pH and create an environment inhospitable to pathogenic bacteria such as *E. coli* and *Salmonella* species. SCFA nourish colonic mucosal cells supplying 60-70% of colonocyte energy needs. Butyrate is the preferred energy source for colonocytes. Studies in animals and humans have found SCFA directly stimulate colonic calcium, magnesium, and potassium absorption, increase colonic blood flow, enhance tissue oxygenation and transport of nutrients, and may be of therapeutic value for various intestinal disorders.^{45,46}

Enhancement of mineral bioavailability

Mineral absorption requires an acidic medium, especially when the minerals are in the form of inorganic salts. Stomach acid is usually sufficient to dissolve mineral salts, but when stomach acid is inadequate mineral salts may not fully dissociate. LAB aid mineral absorption via the production of acidic microenvironments adjacent to the intestinal lining and by generating SCFA that donate protons necessary for mineral absorption. Animal studies have demonstrated that LAB, especially in the presence of a probiotic growth factor like inulin, increase intestinal absorption of calcium, magnesium, potassium and zinc.^{46,47}

Vitamin production

LAB produce small amounts of certain B vitamins, including folates and vitamin B₁₂.⁴⁸ Microbial synthesis of vitamin K in the intestine appears to have nutritional significance in most animal species. Bifidobacteria, streptococci, and enterococci have been shown to produce vitamin K.⁴⁹

Reduction of cholesterol

Studies have shown that some probiotics can lower total serum cholesterol and low density lipoprotein cholesterol.⁵⁰⁻⁵² *In vitro* studies have shown *L. casei* and *L. acidophilus* effectively remove cholesterol from culture media. Researchers postulate that LAB assimilate cholesterol in the gut or deconjugate bile acids disrupting the intestines-to-liver circulation of cholesterol.

Management of inflammatory bowel disease

Inflammatory bowel disease refers to two chronic or relapsing diseases of unknown cause: ulcerative colitis and Crohn's disease. Although these two diseases have some features in common, there are important differences. Ulcerative colitis is an inflammatory disease of the colon. Often the rectum is most severely involved. The colonic mucosa becomes inflamed and develops ulcers. Patients experience diarrhea frequently with blood and mucous in the stool. Crohn's disease most commonly affects the last part of the small intestine (terminal ileum) and parts of the large intestine. However, Crohn's disease can attack any part of the digestive tract. The inflammation of Crohn's disease can extend deeply into the intestinal wall and generally tends to involve the entire bowel wall, whereas ulcerative colitis affects only the bowel lining. Pouchitis is a complication of surgical therapy for ulcerative colitis in which the entire colon has been removed and a pouch made from the ileum has been connected to the anus. Pouchitis refers to inflammation of this pouch. Evidence suggests that inflammatory bowel disease may result from abnormal activation of the mucosal immune system against enteric flora triggering inflammatory mediators.¹ *L. casei* and *L. lactis* have been shown to successfully treat IBD by increasing the gut IgA immune response.⁵³ A mixture of *B. longum*, inulin, and oligofructose reduced inflammatory cytokines and colon inflammation in patients with ulcerative colitis.⁵⁴ Daily intake of *L. rhamnosus* alone or intake of a proprietary combination of *L. casei*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *B. longum*, *B. breve*, *B. infantis* and *S. thermophilus* (VSL) in patients

with pouchitis can provide significant clinical benefit and delay the first onset of pouchitis in patients without symptoms.⁵⁵ *S. boulardii* has been shown to prolong remission and reduce relapses in patients with Crohn's disease.⁵⁶ There is considerable interest in the use of probiotics for inflammatory bowel disease among gastroenterologists.

Amelioration of food allergy

The ability of probiotics to reduce the symptoms of food allergy was noted over 20 years ago.^{57,58} Since then, several well-designed studies have indicated that supplementation with specific probiotic strains are effective for atopic disorders. In infants with atopic eczema and cow's milk allergy, a whey formula supplemented with *L. rhamnosus* GG was shown to significantly improve clinical symptoms and markers of intestinal inflammation.¹⁸ In children with atopic dermatitis, a combination of *L. rhamnosus* and *L. reuteri* proved beneficial.⁵⁹ Consumption of these select *Lactobacillus* probiotics downregulates over-expressed immune responses.

Alleviation of irritable bowel syndrome

Irritable bowel syndrome is a common multifactorial gastrointestinal disorder characterized by flatulence, diarrhea, constipation, and abdominal discomfort and pain. Although challenging to study because of the heterogeneous patient populations, clinical trials with the individual strains *B. infantis*⁶⁰ and *L. plantarum*⁶¹ found that these probiotics effectively reduced irritable bowel syndrome symptoms such as abdominal pain and discomfort, bloating and distention, bowel movement difficulty, and flatulence. In a small pilot study of patients with irritable bowel syndrome, a food elimination diet followed by treatment with a multispecies probiotic preparation (Vital-10® powder) containing *B. bifidum*, *B. infantis*, *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. salvarius*, *L. bulgaricus*, *L. casei*, *L. brevis*, and *S. thermophilus* improved pain, stool frequency, and quality of life scores.⁶² The efficacy of multispecies preparations for irritable bowel syndrome was confirmed by a double-blind, placebo-controlled trial that found a combination of two strains of *L. rhamnosus*, *B. breve*, and *Propionibacterium freudenreichii* spp. *shermanii* reduced symptoms of pain, distension, flatulence, and abdominal gurgling sounds (borborygmi) by over 40%.⁶³

Anti-carcinogenic activity

There is increasing evidence that probiotics have anti-mutagenic and anti-carcinogenic activities in the colon.⁶⁴ Probiotics have been shown to inhibit aberrant crypt (precancerous lesions) formation and tumors in animal models.⁶⁵ Probiotic bacteria may exert anti-carcinogenic effects through a variety of mechanisms. LAB produce organic acids lowering intestinal pH which is strongly associated with a lower incidence of colon cancer.⁶⁶ LAB are able to bind and breakdown dietary mutagenic compounds thereby reducing host exposure. Probiotics may mediate tumor suppression through stimulation of the host immunoprotective response. Probiotic bacteria enhance cytokine production (interferon- γ , interleukin-1 β , tumor necrosis factor- α), macrophage and lymphocyte activation, T- and B-cell proliferation, and antibody production.⁶⁶ Evidence for anti-tumor activity of LAB has been reported in studies using pre-implanted tumor cells in animal models. Feeding cultures of LAB to mice has also been shown to inhibit the growth of injected tumor cells.⁶⁶

PROBIOTICS - Frequently Asked Questions

How do I know I need probiotics?

Probiotics may be used to maintain a healthy, balanced intestinal microflora. Modern diets consisting of highly processed, sterilized foods are deficient in essential microorganisms such as *L. plantarum*, *L. rhamnosus*, *L. casei*, and *L. acidophilus*.⁶⁷ These organisms must be consumed to maintain their needed presence in the gastrointestinal

tract. Infants born by cesarean section and formula-fed infants have disordered intestinal microflora that may have short- and long-term adverse health consequences and may benefit from probiotics.^{68,69} Aging is associated with microflora alterations especially a decrease in the numbers of bifidobacteria.²⁶ Probiotics may reverse age-associated changes in intestinal microbial balance. Probiotics may be taken along with antibiotics, immunosuppressants or other drugs that disrupt the microflora equilibrium. Symptoms that have been extensively studied and shown to be improved by probiotics include antibiotic-associated diarrhea and other types of diarrhea, vaginitis, lactose intolerance, intestinal and vaginal dysbiosis, abdominal distention, flatulence, and constipation. Some probiotics have also been shown to alleviate food allergies and modulate the immune system. When symptoms are present, the need for probiotics is best determined in consultation with a health practitioner experienced in their use.

What probiotics do I take?

Consulting a knowledgeable health practitioner experienced in the use of probiotics is the optimal approach to determine which probiotics may be of most benefit. The appropriate choice of probiotic may be guided by clinical reports and research published in the medical literature. For example, *L. acidophilus*, *L. bulgaricus*, *B. longum*, and *S. thermophilus* have been shown to reduce the risk of diarrhea induced by the antibiotics.¹² At least six rigorous clinical trials have shown that *S. boulardii* substantially decreases the risk of antibiotic-induced diarrhea and protects against *C. difficile*.¹² Rotavirus gastroenteritis in children can be prevented by *L. rhamnosus*, *L. casei*, *S. thermophilus* and *B. bifidum*.⁶ *L. rhamnosus* has been shown to effectively reduce the symptoms of food allergies.^{18,59} *H. pylori*, an organism associated with peptic ulcer disease, is antagonized by a number of lactobacilli including *L. casei*, *L. delbrueckii*, *L. helveticus*, and *L. acidophilus*, as well as by *Bifidobacterium*.^{70,71} For people with irritable bowel syndrome or inflammatory bowel disease a combination of lactobacilli and bifidobacteria appears to offer the most benefit.^{1,31} People desiring to favorably alter their intestinal microflora are best served by a broad combination of *Lactobacillus* and *Bifidobacterium* species.

How will I know if probiotics have helped me?

If symptoms are present, a reduction or resolution of symptoms is the best indication probiotics have helped. Symptoms may take days or even weeks to improve depending on the individual's response to the probiotics and the severity of the underlying condition. If probiotics are used in the absence of symptoms as a general health measure to restore a balanced intestinal microflora, then it may not be apparent that probiotics are helping.

Are there any side-effects from probiotics?

Uncommonly people may experience a worsening of their clinical symptoms following the initiation of probiotics. This is attributed by some practitioners to a "die-off" effect as pathogenic bacteria die releasing toxic cell products. The exact mechanism for a transient worsening in symptoms is unknown, but it does occur. Persistence in taking the probiotics is usually rewarded by an improvement in symptoms. Some individuals may experience gas, abdominal discomfort, and even diarrhea that usually resolve with time. Rarely, probiotics have been associated with opportunistic infections.

Are there tests that can assess my response to probiotics?

With certain exceptions, such as the presence of *C. difficile* toxin or pathogenic microorganisms in the stool, there are no readily available, reliable clinical laboratory tests that can assess a response to probiotics. Many practitioners order stool cultures on the premise that probiotic content in the intestinal tract is reflected in stool count. However, most probiotics are fastidious and not easily cultured from routine stool

samples. Intestinal biopsy and DNA amplification techniques are used currently to demonstrate probiotic colonization of the intestines. A negative stool culture for probiotic species does not indicate lack of colonization, presence, or benefit.

Should I buy a probiotic containing prebiotics?

Prebiotics are carbohydrates that are indigestible by the human intestine and selectively stimulate the activity and growth of certain bacteria in the colon. Most prebiotics are non-digestible chains of 2 to 9 sugar molecules (oligosaccharides). They are commonly found in chicory, asparagus, artichokes, onions, garlic, leeks, and soybeans as well as in human breast milk and cow's milk. Prebiotics have attracted great attention as a way of increasing the number of healthy beneficial commensal bacteria in the intestine. The most extensively used prebiotics include lactulose, oligofructose, galactooligosaccharides, soybean oligosaccharides, and chicory-derived inulin. Some prebiotics, such as lactulose, are synthetic, whereas most other oligosaccharides are natural food components.

After ingestion, prebiotics pass through the small intestine into the colon where they are selectively utilized by the beneficial LAB microorganisms. Numerous studies suggest bifidobacteria prefer short chain oligosaccharides and recent studies suggest oligofructose, soybean oligosaccharides and galactooligosaccharides are the most bifidogenic.^{72,73} Prebiotics can significantly increase the numbers of bifidobacteria in the colon and decrease the populations of pathogenic bacteria such as clostridia, fusobacteria, and Gram-positive cocci. Inulin is a commonly used prebiotic that is not usually associated with the gas and bloating sometimes associated with other prebiotics. Inulin is also more difficult for pathogens to metabolize and unlike some highly processed, long chain fructooligosaccharides less likely to be used as a food source by pathogenic bacteria. Probiotics combined with prebiotics, termed synbiotics, are generally a good choice.

When and how should I take a probiotic?

People often receive contradictory answers to this question. Probiotic labels from different manufacturers may directly contradict each other. Some suggest that the probiotics be taken with meals whereas others recommend between meals. Health professionals are also divided on this topic. Advocates of ingesting probiotics with meals reason that food buffers stomach acid thereby providing protection for the microorganisms. Those who recommend taking probiotics without food usually suggest consuming them with lots of water. The water dilutes the stomach acid and may help move the organisms quickly into the intestines minimizing exposure to acid and bile. Few studies provide direct support for either approach. However, numerous studies have administered probiotics with meals and documented significant benefits. The most prudent approach is to consume probiotics with moderate amounts of food no warmer than room temperature.

If I am taking antibiotics, when should I take a probiotic?

In the past, people were often told not to take probiotics while on antibiotics. The thinking was that the antibiotics would kill ingested probiotics. The trouble with this approach is that it allowed pathogenic microorganisms to proliferate unopposed by beneficial bacteria often resulting in antibiotic-associated diarrhea and other problems. Probiotics should be taken while on antibiotics. The probiotics should be taken at least 1 hour before or 2 hours after ingestion of antibiotics.

How much probiotic do I take?

The answer depends on whether the probiotics are being used for therapeutic reasons or simply to maintain a healthy intestinal microflora balance. In general, a dose of 1 billion colony forming units (CFUs) is required to deliver significant numbers of viable probiotics

to the intestines. There is a trend toward using higher doses. The proprietary probiotic blend VSL#3 comes in packets each containing 450 billion organisms and has been used safely in patients with inflammatory bowel disease. Doses as high as 200 billion CFUs per day have been safe and well-tolerated in patients following liver transplantation. A clinical research study involving the administration of 200 billion organisms daily of a Klaire Labs proprietary blend of 6 probiotic species to kidney transplant patients has been approved by the Western Institutional Review Board and is ongoing.

Do probiotic organisms survive exposure to stomach acid and bile?

Different probiotic organisms have differing sensitivities to stomach acid and bile. *S. boulardii* is not affected by gastric acid. LAB are more sensitive and do not thrive in an excessively acidic or alkaline medium. Some LAB, such as *L. rhamnosus*, are more sensitive than others. Probiotic manufacturers have devised a number of methods to enhance probiotic survival after oral administration. Some manufacturers offer enteric coatings for the probiotics composed of cellulose or, in many cases, synthetic plasticized polymers. Many individuals with environmental sensitivities cannot tolerate the plasticized polymers. One manufacturer has developed an innovative process that coats probiotic microorganisms with vegetable-derived fatty acids. This micro-encapsulation not only shields the probiotics from stomach acid, it protects them from air and moisture and keeps them viable at room temperature. Klaire Labs makes use of a highly purified marine plant extract in an acid-stable technology. The extract is mixed with the probiotics. When exposed to stomach acid it forms a gel-like matrix surrounding the microorganisms protecting them from gastric acid. When delivery of probiotics to the oropharynx, esophagus, and stomach is desirable, probiotics without a gastric acid protective delivery system should be selected.

Do probiotics have to adhere to the intestinal tract to provide a benefit?

Adherence to the intestinal mucosal lining is just one characteristic among many that may, or may not, make an organism useful as a probiotic. Adherence is a property observed under laboratory conditions. There is evidence that normal intestinal microflora do not adhere to intestinal epithelial cells, but live suspended in the intestinal contents.⁷⁴ Adherence is certainly not necessary for a probiotic to provide benefit. There are numerous scientific and anecdotal reports about the therapeutic benefits of yogurt, yet the primary bacteria found in yogurt cultures, *S. thermophilus* and *L. bulgaricus*, are transient microorganisms and do not adhere to the mucosa. Modulation of immune function is a probiotic benefit that clearly does not require mucosal adherence or intestinal colonization. Probiotics are readily taken up by the specialized lymphoid nodules in the walls of the small intestines called Peyer's patches stimulating the production of IgA, cytokines, and other mediators of immune function. Even the administration of dead probiotics has been shown to enhance immune function.⁷⁵

Intestinal mucosal colonization has only been demonstrated *in vivo* for a few probiotic strains. *L. rhamnosus* GG persisted in cultures from rectal biopsies for up to 12 days after oral administration.⁷⁶ Stool culture sensitivity was quite poor even though samples were immediately processed. In one subgroup, *L. rhamnosus* was found in only 20% of final stool cultures compared to 88% of rectal biopsy cultures. In a second study, probiotics were shown to be recoverable from rectal biopsies when dispensed orally to critically ill patients receiving powerful antibiotics.⁷⁷ *L. plantarum* supplementation reduced the populations of pathogenic *Enterobacteriaceae* and sulfite reducing clostridia. *In vivo* studies of probiotic intestinal colonization make two very important points. The first is that routine stool cultures

have a very limited use, if any, in assessing probiotic therapy. The second is that sustained consumption of probiotics is required to maintain colonization and benefit.

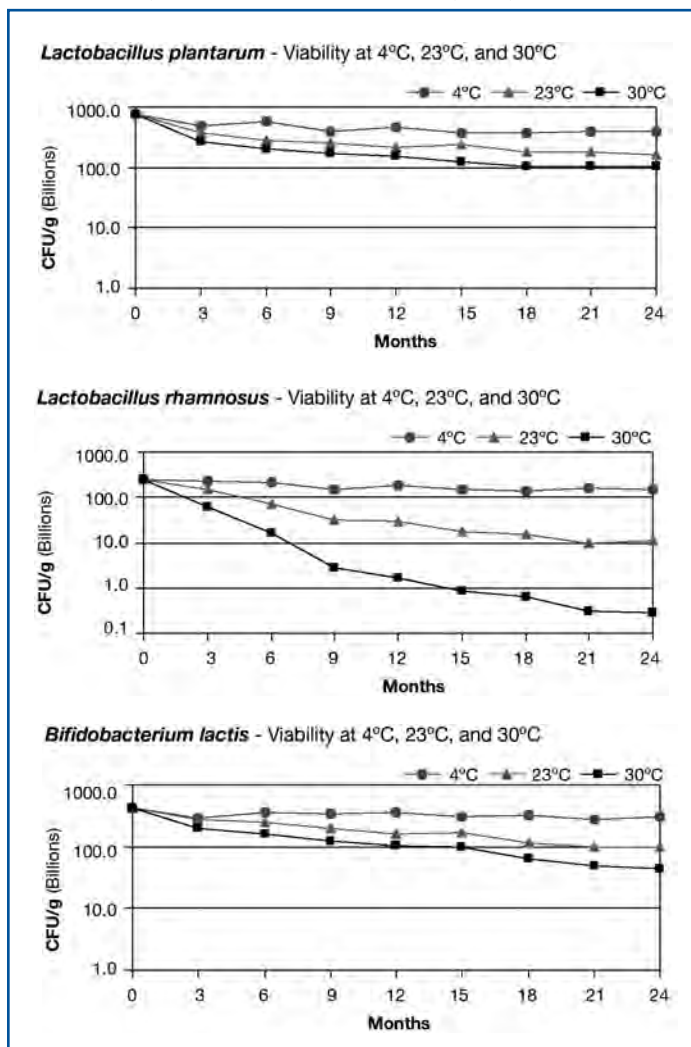
Is freeze-drying of probiotics harmful?

Freeze-drying, or lyophilization, is not harmful to probiotics when done according to strict guidelines. It is an accepted practice that helps ensure long-term probiotic microbial viability. Routine survival studies ensure the probiotics remain viable following freeze-drying. Probiotics that are not freeze-dried, such as liquid probiotics, have a much shorter shelf life even when refrigerated. The short shelf life is due to eventual toxicity of fermentation end-products to the microorganisms. A well-known example is the die-off of yeast that occurs over time during the fermentation of beer and wine. Freeze-drying halts fermentation thereby enhancing microbial viability.

Do all probiotics need to be refrigerated?

In general, it is highly recommended that probiotics be refrigerated to maximally preserve their viability over time. Probiotics that have been microencapsulated with fatty acids are the only exception and do not need to be refrigerated. Probiotics sold in retail stores are often not refrigerated. Consequently, industry and consumer studies have found that 30 to 50% of probiotic products available in retail stores contain significantly less viable microorganisms than claimed on their labels.

Although most probiotics should be refrigerated, they do not spoil or die-off quickly at room temperature. They may be left at room



temperature for days and even weeks without a great loss of viable organisms. They should not be subjected to high temperatures for prolonged periods of time. The graphs illustrate microbial viability for selected probiotic strains over time at 4°C (39°F), 23°C (73°F) and 30°C (86°F) and show that survival is better at low temperatures than high temperatures. At room temperature (23°C), colony counts begin diminishing after one to two months explaining why so many over-the-counter retail probiotic products do not contain the labeled amounts of viable probiotics. The charts also show that certain strains, such as *L. rhamnosus*, are more temperature-vulnerable than others. However, leaving probiotics out at room temperature or even warm temperatures for a few hours or even a few days will only result in small losses of organisms. Although probiotics should be shipped with cold packs, there is no cause for concern or worry if the cold pack has melted by the time of delivery.

Does it matter if probiotics come in plastic or glass bottles?

Probiotics are usually anaerobic organisms, meaning they live in the absence of oxygen. Exposure to air is undesirable and even toxic. Exposure to moisture is potentially more detrimental to freeze-dried probiotics than air. Some people argue that probiotics should be packaged in glass bottles to minimize exposure to air and moisture. However, the difference in permeability between glass and high-density polyethylene (HDPE), a plastic commonly used for bottling, is negligible. Furthermore, once the container has been opened, air and moisture enter the bottle and the relative permeability of glass and HDPE becomes irrelevant. The placement of desiccants inside probiotic containers is a good way to minimize moisture inside the container be it glass or plastic. Bottling probiotics in glass or HDPE containers is equally acceptable.

Is it beneficial to have the supernatant included in the probiotic?

Supernatant is culture medium transformed by the bacteria as they multiply adding a variety of substances to the original medium. Milk is an example of a culture medium; yogurt is an example of a supernatant. At least one company claims that including the supernatant with the bacteria provides additional health properties. Additional benefit to the supernatant is possible, although the evidence is scant. Maintaining the supernatant during production could be detrimental to probiotic survival. The by-products of fermentation may be toxic to the probiotics. The hypothetical benefits of including supernatant seem to be outweighed by the negative impact on probiotic viability.

Can probiotics be used for infants?

Probiotics have been used safely and with benefit in infants. A newborn's intestinal tract is sterile and does not harbor microorganisms. Microbial colonization begins at birth. During the first week of life, *Streptococcus*, *Clostridium*, *Bifidobacterium*, and *Lactobacillus* all vie for predominance. By the end of the first week, bifidobacteria are usually established as the predominant bacteria. Breast-fed infants typically have much higher numbers of bifidobacteria in their intestinal tracts than do formula-fed infants who have higher levels of *E. coli* and other pathogenic coliform bacteria. This is due in part to the presence of bifidogenic substances in breast milk.

Although the newborn intestinal tract is quickly colonized, it is not fully developed. Infants are unable to metabolize a form (isomer) of lactic acid known as D(-). Exposure to D(-)-lactic acid could theoretically lead to D(-)-lactic acidosis, a serious condition.⁷⁸ Some have advocated that probiotic formulations intended for infants should not include D(-)-lactic acid producing organisms such as *L. acidophilus*, *L. brevis*, *L. plantarum*, or *L. bulgaricus*. However, there

are no reports of D(-)-lactic acidosis in infants due to probiotics and D(-)-lactic acid producing probiotics have been used safely in infants.

Clinical trials have demonstrated the benefits and safety of probiotics in infants. In one controlled trial, infants age 3 to 24 months, received an average of 41 million or 3.7 million CFU each of *B. lactis* and *S. thermophilus* per kilogram each day in a standard milk-based formula for an average of seven months. The probiotics were well tolerated and the infants receiving probiotics had adequate growth, less colic or irritability, and a lower frequency of antibiotic use.⁷⁹ In another study, 190 million CFU *B. bifidum* and 14 million CFU *S. thermophilus* per gram of formula reduced the incidence of acute diarrhea and rotavirus shedding during hospital stays.⁸⁰ Two studies have found probiotic supplementation significantly improves infants with atopic eczema.^{18,19} A low dose of 60 million CFU *L. rhamnosus* GG did not decrease the incidence of necrotizing enterocolitis in preterm infants with a gestational age less than 33 weeks.⁸¹ However, a proprietary probiotic formulation containing 10 billion CFU of *L. acidophilus* and *B. infantis* per capsule administered with breast milk significantly reduced the incidence and severity of necrotizing enterocolitis in very low birth weight infants.⁸²

CONCLUSION

Probiotics have been used by people for millennia since the time humans first consumed fermented milk products. Probiotics can be essential for the normal digestive, endocrine and immunological functions of the bowel. They inhibit pathogenic microorganisms and have been used therapeutically to treat a variety of gastrointestinal and even systemic disorders. Probiotics transiently colonize the bowel and, except when used to treat an acute disorder, must be regularly consumed to maintain benefit. Selection of appropriate probiotics, alone and in combination, is best done in consultation with an experienced, knowledgeable health practitioner.

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