

## Probiotics: Considerations for Human Health

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*Evidence for the role of probiotics in maintenance of health or prevention of disease is mounting and is supported in some cases by blinded, placebo-controlled human trials. Today, in an era of antibiotic-resistant pathogens and other looming microbial threats, the value of prevention of infection is recognized. Probiotics may play an important role in helping the body protect itself from infection, especially along the colonized mucosal surfaces of the gastrointestinal tract. Probiotic products are available in many different forms worldwide, including pills, powders, foods, and infant formula. In some cases, general health claims are made that cannot be substantiated for the specific strains and levels being used and consumers must therefore beware.*

**Key Words:** probiotics intestinal health, gastrointestinal tract, antibiotic-resistant pathogens

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### Introduction

Probiotics, live microorganisms administered in adequate amounts that confer a health effect on the host,<sup>1</sup> are emerging as significant dietary ingredients in the field of nutrition. An important function for probiotics is that they influence the populations or activities of host microflora residing in the alimentary canal, the vaginal tract, or the skin. Until recently, the role of gastrointestinal flora in human health was under-appreciated. The concept of colonization resistance (i.e., limiting action of the normal flora on colonization of the bowel by exogenous and endogenous potentially pathogenic microorganisms) has been recognized for decades,<sup>2</sup> but only recently has the significance of this concept resonated with the medical community. The following quote is revealing:

“The toll of human suffering that results from infection and, in particular, from infection with organisms that

can be readily eliminated through the use of antimicrobial agents has engendered a clinical attitude that views those microorganisms as inimical agents of disease to be ruthlessly and utterly eradicated. Yet, the biological relationship between multicellular organisms and the microbial world is better viewed as one of symbiosis than of enmity. Therefore, it follows that preservation of the normal flora, rather than its deletion, can provide the greatest clinical benefit.”<sup>3</sup>

Although the benefits of the normal flora are being acknowledged, the importance of probiotics extends further, because the application of exogenous microbes can also benefit the host. This concept is largely credited to Elie Metchnikoff, an early 20th century Russian scientist awarded the Nobel Prize for his innovative work in immunology. Today a growing industry has developed around the sale of probiotics in food, dietary supplements, and pharmaceutical formats for human and animal use. This article discusses the scientific basis of probiotics and a perspective for their use.

### Why Probiotics?

Why consider the value of probiotics to human health? The gastrointestinal tract and its resident microbes have far-reaching implications for health. The normal microflora limits the ability of potential pathogens to infect. Boosting the body’s ability to resist infection prevents morbidity, decreases antibiotic use (and possibly the spread of antibiotic-resistant pathogens), and decreases the sequelae from some primary infections, which are rare, but nonetheless can be serious. A breakdown of tolerance to the intestinal flora is thought to be a key step in the development of inflammatory disorders of the intestine (e.g., Crohn’s disease and ulcerative colitis) for which there is no cure and for which treatment can be as severe as surgical removal of the colon. Errant activities of some intestinal bacteria are thought to contribute to the generation of carcinogens, which may play a role in the genesis of colon cancer. Exposure of the immune cells of the intestinal tract to the right types of microbes in infancy may be important to the prevention of allergy development later in life. Taken together, these facts suggest that intervention at the level of microflora may be important to health.

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**Table 1.** Published Definitions of Probiotics

Published Definition	Reference
Substances produced by microorganisms that promote the growth of other microorganisms	4
Organisms and substances that contribute to intestinal microbial balance	5
A live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance	6
A viable mono- or mixed-culture of microorganisms that, applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora	7
Living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition	8
A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract	9
A preparation of or a product containing viable, defined microorganisms in sufficient numbers, that alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host	10
Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host	1

### Probiotic Definitions

Although the concept of probiotics was introduced in the early 20th century, the term was not coined until the 1960s. The definition of the term has evolved through the years (Table 1). Characteristics of the definition proposed by an Expert Consultation in an FAO/WHO report<sup>1</sup> follows.

Probiotics must be alive. Although it is recognized that dead cells may mediate physiologic benefits, a different term should refer to these agents.

Probiotics are administered. A misuse of the term equates “probiotic” with native “beneficial” flora (presumably lactobacilli and bifidobacteria). Although native beneficial flora may be isolated, purified, characterized, evaluated, and ultimately used as probiotics, in their native state they are not probiotics.

Probiotics must deliver a measured health benefit, substantiated by studies conducted in the target host. Not specified in this definition is that some benefits may be physiologic markers presumed to be linked to a health benefit, such as lowering cholesterol. If used to substantiate efficacy, such biomarkers should be validated.

Probiotics needn't be restricted to food applications or oral delivery. Probiotics used as pharmaceuticals or as topical agents are not excluded from this definition.

A definition of probiotics shouldn't limit the mechanism of action. A definition stating that a probiotic must survive gastrointestinal tract transit or have an impact on normal microflora is too restrictive, considering the wide range of proposed mechanisms that mediate probiotic function. The delivery of lactase by, for example, *Streptococcus thermophilus*, to the small intestine is recognized as probiotic activity, even though *S thermophilus* does not survive intestinal transit.

Not included in this definition are stipulations for safety or for use of defined strains. Safety is implied; the

definition states that the result of the probiotic is a health effect. In practice, a probiotic product should comprise one or more defined strains. It is scientifically untenable to validate probiotic function and monitor probiotic impact on a preparation of microbes of unknown composition. Probiotic activities have been deemed largely strain-specific, so definition to the strain level is important. Deposit of a probiotic strain into an internationally recognized culture collection is recommended.

It is important to have an agreed upon definition by the scientific community, even in the absence of regulatory definitions. In the absence of a definition, consumers cannot know what to expect from a product carrying this designation and there can be no common understanding among scientists about appropriate use of the term.

### Probiotic Effects and Mechanisms

The impact of probiotics on diverse human physiologic endpoints has been tested. Recent reviews describe details of many human studies testing the efficacy of probiotics.<sup>11–14</sup> Table 2 lists the targets that have been evaluated. Although hundreds of publications on probiotics can be found in the scientific literature, only a few strains acting on a few clinical targets enjoy the status of having “established effects.” This judgment is made when considering the totality of the evidence (in vitro, animal, and human data) supported by a plausible mechanism of action. The key to comprehending that a wide range of physiologic parameters can be influenced by probiotics is understanding the diverse effects of colonizing microbes.

As with research on any dietary component, some qualifications must be kept in mind when considering the body of published literature. First, generalizations about probiotics can be misleading. Different strains or combinations of strains cannot be expected to have the same

**Table 2.** Endpoints in Human Subjects for Probiotic Studies

Target	Proposed Mechanisms	Reference
Allergy (atopic eczema, milk allergy, rheumatoid arthritis)	Translocation/barrier effect	15–18
Cariogenicity	Alteration of populations, activities, or ability to adhere to teeth of the oral microflora	19
Carcinogenicity, mutagenicity, tumor	Mutagen absorption Immune stimulation Inhibition of carcinogen-producing intestinal microflora	20, 21
Cholesterol reduction	Deconjugation of bile acids	22
Diarrhea (antibiotic-associated, rotavirus, <i>C difficile</i> colitis, travelers, community acquired)	Competitive exclusion Translocation/barrier effect Enhancement of immune response	23
Endotoxemia associated with alcoholic liver disease	Inhibition of endotoxin-producing intestinal microflora	24
<i>Helicobacter pylori</i>	Antipathogen activity	26
Hypertension	Cell components or fermentation-derived peptides acting as ACE inhibitors	27
Immunomodulation (immune status, vaccine response)	Interaction with immune cells or cell receptors leading to increased phagocytic activity of white blood cells, increased serum IgA after antigen exposure, increased proliferation of intra-epithelial lymphocytes, regulation of the Th1/Th2 balance, induction of cytokine synthesis	28
Irritable bowel syndrome; general gastrointestinal tract symptoms (constipation, non-pathogen-induced diarrhea, bloating, gas, cramping, gut-caused halitosis)	Alteration of populations or activities of intestinal microflora	29
Inflammatory bowel diseases, ulcerative colitis, Crohn's, pouchitis.	Down-regulation of inflammatory response	30
Kidney stones	Alteration of gut flora influencing oxalate degradation	31
Lactose intolerance	Delivery of microbial lactase to small intestine	32
Small bowel bacterial overgrowth	Antimicrobial activity, competitive exclusion	33
Vaginosis, urinary tract infections	Antipathogen activity, competitive exclusion	34

Note: At least one double-blind, placebo-controlled human trial supports many of these targets, although some trials may have been conducted in a small number of subjects. Some targets are only supported by in vitro or animal studies. Reviewed in Mercenier et al.<sup>14</sup>

effect. Second, mechanisms are often not understood. In the absence of a credible hypothesis for the effect, studies documenting health effects must be considered preliminary. Third, effects should be evaluated with reference to the totality of the evidence. Private industry groups with a clear objective to establish functionality of their commercial products often fund studies. These studies should be confirmed in independent laboratories. Furthermore, negative results are rarely published. Therefore, the totality of the evidence is not made available for scientific consideration. The willingness of editors of scientific publications to publish negative results, especially of well-conducted human studies, should be encouraged.

### Probiotic Selection

The concept of “proper strain selection” for identification of effective probiotics figures prominently in the literature. The best known commercial strains are accompanied by descriptions of the scientific processes by which the strains were selected from among many other strains deemed less useful. Interestingly, however, the multitude of characteristics or tests (e.g., human origin, acid tolerance, bile resistance, adherence to human intestinal cell lines, bacteriocin production, and colonization, among others) used by most investigators to select these strains have not been validated as important criteria for probiotic functionality. For example, controlled studies with

isogenic mutants with mutated capacity for the attribute in question should be conducted where possible. For many in vitro selection criteria, there is no scientific substantiation beyond the assertion that “it stands to reason” that the attributes deemed essential for probiotic function are even important.

This is not meant to discourage careful laboratory analysis of strains for probiotic use. It is intended to highlight the limitations of some selection criteria and point out the need to restrain from overextending the meaning of such evaluations. Certain in vitro analyses are critical to responsible probiotic characterization, such as thorough taxonomic evaluation (using DNA-based and phenotypic techniques), strain identity patterns (using a combination of phenotypic, morphologic, and DNA-based techniques), safety assessment, and metabolic, enzymatic, and physiologic capabilities. Until those characteristics are proven to be important to in vivo function by controlled studies, however, their importance should not be assumed.

### Probiotic Strains and Products

Probiotics are available in products such as food, dietary supplements, and drugs. In the United States, there are no approved probiotic drugs for human use, although one product, Preempt, which was developed by the United States Department of Agriculture, is available as an animal drug (the microbial preparation is used on newly hatched chicks to help prevent colonization by pathogens). Probiotics sold as pharmaceuticals are available in other countries. In the United States, food products containing probiotic bacteria are almost exclusively dairy products. Approximately 80% of the yogurt manufactured in the United States contains *Lactobacillus acidophilus* added as a probiotic. Some products also contain *Bifidobacterium* strains. In addition to yogurt, probiotic bacteria are added to milk, some cottage cheeses, and a few niche products such as rice milk. A fermented milk beverage, Actimel (Dannon, Tarrytown, NY) is a distinctive product in the United States available nationwide at a natural foods grocery chain. It contains  $10^{10}$  *Lactobacillus casei* per 100-mL serving. Actimel is labeled as a dietary supplement and is unique for a fermented dairy beverage in the United States in that it lists the probiotic level ( $10^{10}$ /serving) on the label. Outside the United States, probiotic-containing food products include cheese, toddler formula, and juices. Products such as breakfast cereal and nutrition bars containing probiotics are not currently available, but they present attractive possibilities for future probiotic products. Recently, the United States Food and Drug Administration indicated that it had no questions regarding the effort of Nestle (Lausanne, Switzerland) to affirm the generally recognized as safe (GRAS) status of *Strepto-*

*coccus thermophilus* and *Bifidobacterium lactis* for use in formula for infants greater than 4 months of age; this opened the door for the sale of probiotic-containing formula in the United States.

The manufacturer often determines strategies for formulation of U.S. dairy products with probiotic bacteria. The U.S. standard of identity for yogurt requires that yogurt be made with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as fermenting starter cultures, but no levels are specified. Probiotic bacteria such as *Lactobacillus acidophilus* or *Bifidobacterium* spp. are allowable in yogurt and milk, but no minimum levels are required. Some commercially used strains are listed in Table 3. Culture manufacturers recommend approximately  $10^6$  probiotic bacteria per gram of yogurt and unfermented acidophilus milk. Even if this formulation recommendation is followed, there is no guarantee that the product contains this level at the time of consumption. Viable counts may fall below these levels if the bacteria used are not biologically stable in the particular food formulation.

Recognizing the importance of assuring consumers of the presence of live cultures with possible beneficial effects, the National Yogurt Association (McLean, VA) established a “Live Active Culture” seal that helps consumers identify yogurt containing live, active cultures; this seal can be used by any yogurt manufacturer on labels of yogurts meeting minimum standards. Use of the seal requires refrigerated yogurt to contain  $10^8$  viable lactic acid bacteria per gram at the time of manufacture. The seal can also be used on frozen yogurts containing  $10^7$  viable lactic acid bacteria per gram at the time of manufacture. However, these standards do not pertain to each species or strain listed on the label. Therefore, counts of the starter culture *S thermophilus* may meet the standard, but counts of *L acidophilus* or *Bifidobacterium* may not. The consumer therefore suffers from a lack of clear labeling standards for probiotic-containing products.

Dietary supplements are another source of probiotics. These products, usually available in pill or capsule form, are produced by approximately 80 different companies in the United States. The product diversity is much greater than what is found in dairy products and this market segment is growing faster than the probiotic-containing dairy segment in the United States. Whereas dairy products containing probiotics generally contain *Lactobacillus* or *Bifidobacterium* species, dietary supplement (or biotherapeutic) products on the worldwide market also contain *Enterococcus* species, *Bacillus* species, *Escherichia coli*, and/or *Saccharomyces*. Unfortunately, the lack of standards (whether imposed by government or industry) has resulted in products that are not labeled properly.<sup>35</sup> Some of this mislabeling is reasonably harm-

**Table 3.** Examples of Some Commercially Available *Lactobacillus* and *Bifidobacterium* Probiotic Strains

Strain	Source
<i>L acidophilus</i> NCFM®	Rhodia, Inc. (Madison, WI, USA)
<i>L acidophilus</i> DDS-1	Nebraska Cultures, Inc. (Lincoln, NE)
<i>L acidophilus</i> SBT-2062*	Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)
<i>L acidophilus</i> LA-1 (or LA-5 in Europe)	Chr. Hansen, Inc. (Milwaukee, WI, USA)
<i>L acidophilus</i> NCFB 1748	Arla (Stockholm, Sweden)
<i>L paracasei</i> Shirota* (YIT 9018)	Yakult (Tokyo, Japan)
<i>L paracasei</i> Immunitas (DN 014001)	Danone (Paris, France)
<i>L paracasei</i> F19	Arla
<i>L johnsonii</i> La-1	Nestec Ltd. (Lausanne, Switzerland)
<i>L paracasei</i> CRL 431	Chr. Hansen, Inc.
<i>L plantarum</i> 299V	Probi AB (Lund, Sweden)
<i>L reuteri</i> SD2112	Biogaia (Raleigh, NC)
<i>L rhamnosus</i> GG* (ATCC 53103)	Valio Dairy (Helsinki, Finland)
<i>L rhamnosus</i> 271	Probi AB
<i>L rhamnosus</i> LB21	Essum AB (Umeå, Sweden)
<i>L rhamnosus</i> DR20 (HN001)	Fonterra Cooperative Group Ltd (Auckland, New Zealand)
<i>L salivarius</i> UCC118	University College (Cork, Ireland)
<i>Bifidobacterium lactis</i> Bb-12	Chr. Hansen, Inc.
<i>B longum</i> BB536*	Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)
<i>B longum</i> SBT-2928*	Snow Brand Milk Products Co., Ltd.
<i>B breve</i> strain Yakult*	Yakult
<i>B lactis</i> DR10 (HN019)	Fonterra Cooperative Group Ltd.

\* Strains have been granted status as active ingredients in Foods for Specified Health Use (an approval as a documented functional food by the Ministry of Health) in Japan.

less. However, persistent use of the term “*Lactobacillus sporogenes*,” when in fact the organism is a *Bacillus* species, perhaps *coagulans*, is not a benign error.<sup>36</sup> Some products contain *Enterococcus* but do not list this on the label. *Enterococcus* species have been associated with transferable antibiotic resistance genes and are a leading source of nosocomial infections in the United States.<sup>37</sup> Franz et al.<sup>38</sup> commented that in the past, *Enterococcus* species were considered “. . . harmless commensals with low pathogenic potential,” but today they “. . . may be considered opportunistic pathogens.” Although infection resulting from *Enterococcus* consumption in foods or probiotic supplements has not been reported, consumers should be properly informed about product contents.

Labeling of U.S. food or dietary supplement products must conform to U. S. food regulations. In short, both foods and dietary supplements are allowed to make statements relating the food or supplement to the normal functioning of the human body (structure/function claims), but statements regarding their role in the prevention, treatment, cure, diagnosis, or mitigation of disease are expressly forbidden because these claims are limited to use on drugs. Manufacturers of dietary supplements must notify the U.S. Food and Drug Administration of structure/function statements they intend to make on products, but premarket approval of such statements is not required. Examples of some structure/function

statements used on U.S. probiotic dietary supplement products are shown in Table 4.

The perception of probiotic products in Europe (especially northern Europe) and Asia differs from that in the United States. Currently, there is no harmonized legislation in Europe for the use of probiotics, but in general, little can be legally claimed. In Japan, there is legal recognition of functional foods (Foods for Specified Health Use, or FOSHU) and several probiotic products and strains have been granted FOSHU status by the Japanese Ministry of Health. Recently, the FAO and WHO jointly convened an expert consultation on foods containing probiotics. The report has been posted.<sup>1</sup> Guidelines for the safe use and labeling of probiotics developed as a follow-up to the expert consultation are also available.<sup>42</sup>

### Safety

One hundred percent safety can never be guaranteed. However, many species of lactobacilli are integral to the production of fermented foods and have been consumed safely as part of these foods for millennia. In addition, both bifidobacteria and some species of lactobacilli are normal, nonpathogenic inhabitants of the human intestinal tract. Intestinal lactobacilli (some species not normally associated with fermented foods) and bifidobacte-

**Table 4.** Examples of Probiotic Dietary Supplement Product Claims in the United States

Claims	Product	Manufacturer	Source
Supports immune function Supports intestinal detoxification and promotes normal bowel function Supports healthy intestinal balance	All-Flora Live Cells	New Chapter Inc., Brattleboro, VT	39
Helps to create a favorable environment for the growth of beneficial flora, which dramatically influences metabolism and physical well being Lowers pH of the intestine Manufacturers specific B-vitamins Promotes immune function and overall health	Spectra Probiotic	NF Formulas, Wilsonville, OR	39
When taken daily, helps fortify your body's natural defenses and helps keep your body at its best Clinically proven at major U.S. and European institutions to maintain a healthy intestinal tract and strengthen the body's natural defenses	Acidophilus	Cell Tech, Klamath Falls, OR	39
	Bifidus	Cell Tech, Klamath Falls, OR	39
	Actimel	Dannon, Tarrytown, NY	40
	Culturelle	CAG Functional Foods, Omaha, Nebraska	41

ria have been used in dietary supplements for decades, with an overwhelming record of safe consumption. The conclusion of a recent review of safety of lactobacilli and bifidobacteria is that there is essentially no risk with the oral consumption of lactobacilli or bifidobacteria by healthy people.<sup>9</sup> In patients with certain underlying diseases or conditions, however, some caution should be exerted. In fact, native lactobacilli and bifidobacteria have been documented as causative agents of infections in patients with compromised health.<sup>43–45</sup> Some metabolic end products may be a concern. Specifically, D-lactic acidosis can result in people with short bowel syndrome if the probiotic produces D-lactic acid from sugar metabolism.<sup>46</sup> This situation can be avoided by choosing probiotics that exclusively produce the L-isomer of lactic acid. There are only two documented cases of association between probiotic *Lactobacillus* consumption and infection, and this association was correlative, not causal. Two different probiotic preparations containing *L rhamnosus* were involved with adverse effects: a liver abscess in a 74-year-old diabetic woman with hypertension<sup>47</sup> and endocarditis in a 67-year-old undergoing tooth extraction with a history of mitral valve prolapse.<sup>48</sup> These reports suggest that lactobacilli can behave opportunistically, albeit extremely rarely.

In a discussion of safety of probiotics, however, one must consider the broad range of microbes beyond the lactobacilli and bifidobacteria that are used as probiotics. Strains of *Enterococcus*, *Bacillus*, and *Escherichia* should be evaluated comprehensively for safety. Even the yeast, *Saccharomyces*, can be problematic under certain circumstances. Hennequin et al.<sup>49</sup> documented 13 cases of fungemia owing to vascular catheter contamination in hospitalized patients consuming *Saccharomyces* as a probiotic; this was likely due to cross contami-

nation of catheters when the probiotic was administered. *Enterococcus* species are a leading cause of nosocomial infections worldwide and are an important reservoir of transferable antibiotic resistance genes. A group of experts advised that *Enterococcus* not be used as a probiotic in foods.<sup>1</sup> *Bacillus* infections linked to probiotic consumption include three reports<sup>50–52</sup> detailing seven cases of *B subtilis* bacteremia, septicemia, and cholangitis, all in patients with underlying disease.

In general, considerations for safety should include knowledge of history of safe use under the recommended route of administration (some non-oral applications have been proposed, e.g., intravaginal), the health status of the consumer, the frequency of association of species with infection, the likelihood of production of potentially deleterious metabolic end-products (including D-lactic acid), association with transferable antibiotic resistance, sensitivity to therapeutic antibiotics (for use if an infection does occur), and relation to species that produce hemolysins, mammalian toxins, or other virulence factors. The documentation of safety is an important reason why probiotics should be defined strains, properly classified with regard to taxonomy, and properly named according to current conventions of bacterial nomenclature.

### Future Research

Probiotics hold much promise. However, the data supporting their use is emerging and has limitations. Some key areas for research include:

- Definition of physiologically relevant and validated biomarkers useful for assessing the impact of probiotics on human health
- Follow-up of preliminary studies with confirmatory

studies, preferably in independent labs or clinical settings

- Establishment of mechanisms of action
- Dose-response studies
- Definition of the active principle in probiotic product (viable cells, fermentation end-products, cell wall components, etc.)
- Validation of in vitro assays; tests using isogenic strains that differ only in one parameter (i.e., isolate a non-adhering mutant or non-bacteriocin-producing strain and determine if its physiologic effect is any different from parent strain) would be useful in this regard
- Epidemiologic study of probiotic effects, possible in populations with a significant penetration of probiotic use

Some novel applications and research with probiotics have emerged recently. Patton et al.<sup>53</sup> genetically engineered a non-pathogenic strain of *E coli* to express galactosyl transferase derived from a *Neisseria* strain. This enzyme attached galactosyl residues that mimicked the receptor for shiga toxin onto the lipopolysaccharide core on the surface of the host *E coli*. The engineered *E coli* was capable of titrating the shiga toxin from a liquid medium. Mice treated with a lethal dose of shiga toxin-producing *E coli* survived the infection.

Another area in development takes advantage of the gut mucosal surface, a key site for development of immune response. Delivery of antigens to the gut mucosal surface via engineered lactococci and lactobacilli has been investigated. These live microbes would essentially serve as oral vaccines, with targets such as rotavirus,<sup>54</sup> foot-and-mouth disease virus,<sup>55</sup> and bovine coronavirus.<sup>56</sup> In another approach using live microbes, Steidler et al.<sup>57</sup> engineered a strain of *Lactococcus lactis* to secrete the anti-inflammatory cytokine interleukin-10. Intra-gastric administration of this bacterium caused a 50% reduction in colitis in a mouse model of chemically induced colitis, suggesting a new therapeutic approach to the treatment of inflammatory bowel diseases. Although these strategies require the use of recombinant genetic techniques, the successes of the approaches suggest great potential for the novel use of live microbes to improve health.

The ability to analyze bacterial communities has undergone tremendous advancement with the application of polymerase chain reaction technologies and appropriate DNA probes. One technique, terminal restriction fragment polymorphism (TRFP), is especially valuable for determining the impact probiotics have on intestinal microecology.<sup>58</sup> Instead of relying on culture techniques for determining perhaps six to ten large groups of bacteria in the intestine, this technique allows semiquantitative resolution of dozens of native bacterial species

comprising ~99.99% of the bacteria in a community. If applied to fecal or intestinal samples, the changes in bacterial communities detected by TRFP can be indicative of the impact of probiotics.

Lastly, as with virtually all branches of biology, genomic sequencing and subsequent functional genomic efforts will have a great impact. Knowledge of the genome of probiotic bacteria will greatly enhance efforts to elucidate mechanisms and improve or design strains for maximum physiologic effectiveness. Currently, the genomes of several lactobacilli are fully sequenced (although not all sequences are in the public domain) and additional species of both lactobacilli and bifidobacteria are in progress.

## Conclusion

Much remains to be learned about the role of probiotics in human health. This is clearly an emerging area of science and one requiring confirmation of efficacy and mechanisms of action in controlled studies. However, the cumulative information that does exist has begun to establish a credible hypothesis about the role of probiotics in enhancing human health. The ongoing multidisciplinary approach to exploration of this field will be invaluable to this effort.

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