Impact of Probiotics on Colonizing Microbiota of the Gut

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Abstract: Although precise mechanisms responsible for all demonstrations of probiotic health benefits are not known, many lines of evidence suggest that probiotics function through direct or indirect impact on colonizing microbiota of the gut. Probiotics can directly influence colonizing microbes through multiple mechanisms, including the production of inhibitory compounds (bacteriocins, short chain fatty acids, and others), by producing substrates that might promote the growth of colonizing microbes (secreted exopolysaccharides, vitamins, fatty acids, sugars from undigested carbohydrates and others), and by promoting immune responses against specific microbes. Indirectly, probiotics can influence colonizing microbes by inhibiting attachment through stimulated mucin production, reinforcing gut barrier effects, and downregulation of gut inflammation, thereby promoting microbes that are associated with a healthier gut physiology. Although the value of targeted changes in populations of gut bacteria is a matter of debate, increased levels of *Bifidobacterium* and *Lactobacillus* in the gut correlate with numerous health endpoints. Microbiota changes due to probiotic intake include increased numbers of related phylotypes, decreasing pathogens and their toxins, altering bacterial community structure to enhance evenness, stabilizing bacterial communities when perturbed (eg, with antibiotics), or promoting a more rapid recovery from a perturbation. Further research will provide insight into the degree of permanence of probiotic-induced changes, although research to date suggests that continued probiotic consumption is needed for sustained impact.

Key Words: probiotic, microbiota, bacterial communities, *Lactobacillus*, *Bifidobacterium*

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Probiotics are living microorganisms, which when administered in adequate amounts confer a health benefit on the host.[1] Although this definition does not stipulate a mechanism of probiotic action, the means by which probiotics achieve their effects is often due to a direct or indirect impact on the populations or activities of the colonizing microbiota.[2] However, the nature of such interactions is not always known, especially in humans. But a clear sense of how probiotics impact colonizing microbiota will shed important light on how they function and how we might optimize their benefits. No doubt, findings generated from global projects investigating the nature of the human microbiome will provide critical baseline information and methodologies on which the probiotic field can build.

The means with which probiotics impact the microbiota populations or activities are numerous. Some probiotics have direct action on microbes through production of bacteriocins and primary and secondary fermentation end products, which can inhibit pathogens or provide a means to influence commensals.[3,4] Probiotics can inhibit adherence of pathogens through stimulation of epithelial cell mucin production.[5] Probiotics can reinforce the tight junction between intestinal epithelial cells, reducing the likelihood of bacterial translocation.[6] Probiotics can use substrates throughout the gut, or deliver enzymes that can break down substrates in situ (eg, lactase), thereby influencing the available substrates for commensal bacterial growth (or overgrowth).[7,8] Finally, probiotics interact in diverse ways with immune system components.[9] A stimulation of antimicrobial immune activity can reduce populations of certain microbes; a downregulation of gut inflammation can promote microbes that are associated with a healthier gut physiology.

This review will explore the current state of research on the impact of probiotics on gut microbiota.

WHAT CHANGES IN THE GUT MICROBIOTA ARE BENEFICIAL CHANGES?

The taxonomic composition of an "ideal" microbiota, if it exists, remains to be defined. Recently, Arumugam et al[10] found that microbial communities of feces clustered into 3 types (enterotypes) irrespective of geographical origin, body mass index, age, or gender of study subjects. These results comprise evidence that there is not one, target, healthy microbiota species composition, but "a limited number of well-balanced host-microbial symbiotic states,"[10] which will likely be refined with further research. How much overlap exists among these 3 enterotypes with regard to composition or molecular functions remains to be determined. Importantly, the investigators concluded that these microbial clusters are probably not determined simply by diet, age, or body mass index, even though functional microbial markers for age and body mass index were identified.

Although the healthy microbiota remains to be defined, there are numerous diseased states associated with a disturbed gut microbiota (Table 1). In most cases, it is not know if the disturbed microbiota is causal or correlative with the disease. But the fact that disturbed microbiota may play a role in the onset or development of certain diseases leads to the hypothesis that interventions that can return the microbiota to a healthier state may mitigate the disease. Administration of properly selected probiotics may be such an intervention.

The impact of modern lifestyles on the composition of microbiota is an important research question. One hypothesis states that the colonizing microbiota of healthy people from developed regions of the world may not reflect a

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TABLE 1. Diseases and Disorders Associated with Human Gut Microbiome Aberrations (Adapted from[26])

Disease	Reference
Atopy and asthma	27
Celiac disease	28
Colon cancer	29
Type I diabetes	30
Type II diabetes	31
HIV infection	32
Inflammatory bowel disease	33–35
Irritable bowel syndrome	36–37
Gastroenteritis	38,39
Necrotizing enterocolitis	40
Obesity	41
Rheumatoid arthritis	42

HIV indicates human immunodeficiency virus.

healthy ideal. Profound changes in diet and lifestyle conditions began with the introduction of agriculture and animal husbandry approximately 10,000 years ago. Certainly, great differences in diet (particularly regarding nondigestible dietary substances such as fibers that when present fuel colonic fermentation), hygiene, insulation from environmental microbial exposure, exposure to antibiotics, and other factors, likely shape the microbial community differently than what would have occurred over the course of all but the past approximately 70 years of human existence. So perhaps "normal" for the modern day, healthy person, is not equivalent to ideal. One recent study [11] compared the fecal microbiota of children from Italy—who ate a typical western diet high in animal protein, sugar, starch, and fat, and low in fiber, to that of a rural African village of Burkina Faso—who ate a diet high in starch, fiber, and plant polysaccharides. The African diet is thought to be similar to the diet of early man. Distinct differences in the microbiota populations, level of diversity, short chain fatty acid levels in feces, and the ability of resident microbes to metabolize fibers and starches were observed. These microbiota differences were hypothesized to be largely due to the different diets, although the almost 2-fold greater total calorie consumption by the Italian children compared with the African children may be a confounding factor in this interpretation.

Altering the gut microbiota to reduce microbes linked to pathogenic activity may be a reasonable goal for changing the gut microbiota, but the meaningfulness of magnitudes of reduction is not always apparent. Many factors may impact the risk associated with the presence of a microbial pathogen, such as host susceptibility and virulence of the specific strain of pathogen present.

Aside from specific, known pathogens, other microbiota changes have been proposed as beneficial. For example, the concept of prebiotics is predicated on improving the populations or activities of beneficial members of the native colonizing populations,[12] and probiotics are touted for their ability to balance the microbiota. Historically, the focus of prebiotics has been on increasing levels of *Bifidobacterium* or *Lactobacillus* species. This thinking has not been universally accepted,[13] although there are compelling data correlating the increase of *Bifidobacterium* and improved health.[12] Critics contend that causality has not been established, and that *Bifidobacterium* may be a biomarker of health and not the cause. However, the *Bifidobacterium* genus meets the characteristics proposed for a beneficial bacterium, including saccharolytic metabolism, ability to reduce colonic pH in vivo, nonpathogenic or toxinogenic, and devoid of transferable antibiotic resistance (Glenn Gibson, personal communication). Surely, as the puzzle of the human microbiome is unraveled, the list of putatively beneficial microbes will expand. Microbes such as *Faecalibacterium prausnitzii*, an anti-inflammatory commensal bacterium,[14] *Eubacterium,* and *Roseburia* have been proposed, suggesting that a focus on *Bifidobacterium* may be over simplifying the situation.

Another way that probiotics may impact the gut microbiota might be by protecting it from disruption. Such an impact could be reflected after perturbation in a more rapid return to normal, or reducing the degree of gut microbiota disruption (Fig. 1). Underlying this concept is

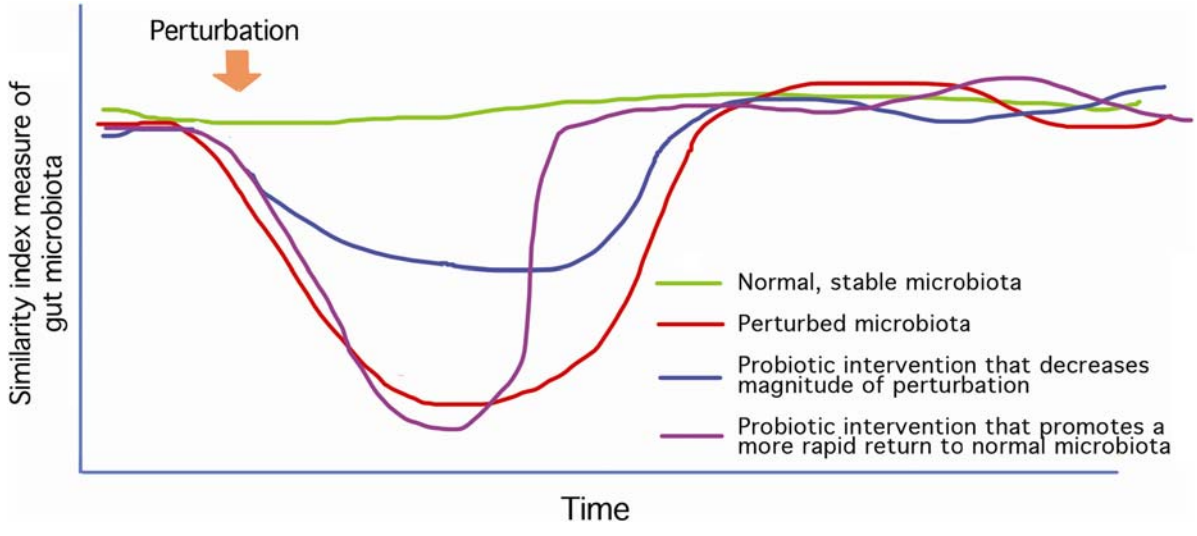

FIGURE 1. Probiotic intervention that decreases the magnitude of change or promotes a more rapid return to normal in a perturbed gut bacterial community. (Sanders et al. In Press). Reprinted with permission from Gut Microbes.

the principle that maintaining stability of a healthy individual's microbiota population is beneficial, without addressing the specific microbial populations that are present. For example, Kajander et al[15] conducted a 6-month feeding study on 42 irritable bowel syndrome (IBS) patients. The probiotic used was a blend of 4 strains, *Lactobacillus rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. shermanii JS, and *B. breve* Bb99. A few specific changes in microbes were detected with quantitative polymerase chain reaction (*Clostridium* and *Ruminococcus* groups), but more interesting was the observation that probiotic supplementation promoted stabilization of the microbiota (as revealed with an increased overall similarity index). A similar finding was reported in a study on patients with Japanese cedar pollinosis.[16]

PROBIOTIC IMPACT ON SPECIFIC COMPONENTS OF MICROBIOTA

Over past decades, numerous studies have tracked the impact of probiotic feeding on microbiota. These studies generally aimed to determine: if the probiotic could survive intestinal transit (a criterion potentially important to its ability to impact intestinal health); if any probiotic-induced changes in populations of intestinal microbes could be measured; and the duration of any observed changes in microbe populations. These studies were primarily done on fecal samples, and in some cases, these studies reported changes in bacterial metabolites, such as short chain fatty acids, amines, indoles and others. Often, these studies relied on culture-dependent approaches that looked at only targeted members of the gut microbial community. As estimates are that between 75% and 95% of gut microbes are not recovered by culturing,[17] these older studies are limited in their scope.

General conclusions from these studies are: probiotic survival through the intestine is dose-dependent and strain-dependent; probiotic microbes in general do not persist past 1 to 2 weeks once feeding has stopped; and aside from the transient, documented changes in the genus of the fed probiotic, the impact of probiotic feeding on the populations of other microbes that were recovered from feces is not consistent among studies and the degree of documented change, although statistically significant, was often not of large magnitude (Table 2). In some cases, feeding *Lactobacillus* or *Bifidobacterium* probiotics did result in changes of specifically assayed fecal bacteria, but those changes were not consistent among different studies, and are likely dependent on factors that differ among studies, such as probiotic, duration of feeding, and time of sampling (during product administration or during follow-up). For example, reduction of populations of enterobacteria and clostridia was demonstrated by Fujiwara et al,[18] but Rinne et al[19] showed an increase of *Clostridium* immediately after a 6-month administration but a reduction after 18-month follow-up with no consumption. Culture-independent methods targeted toward bacterial community analysis will provide a broader understanding of how probiotic feeding impacts populations of the microbiota.

TABLE 2. Summary of Key Findings from Culture-dependent Assessments of the Impact of Probiotics on Colonizing Microbiota

Effects depend on strain, dose, and methods used
Transient increases in the genus, species, or strain of the fed probiotic strain are often observed in feces of patients
The fed probiotic is often not isolated 1 to 4 weeks after feeding has stopped (a few exceptions)
Changes in fecal populations of nonprobiotic species and genera are sometimes not observed and are not consistent among studies
Changes in biochemical parameters are sometimes observed, including changes in short chain fatty acid profiles, ammonia, amines, pH, phenols, p-cresol, and enzymatic activities
Reduction in numbers or virulence of pathogens or levels of toxins is sometimes observed

IMPACT OF PROBIOTICS ON THE BROADER BACTERIAL COMMUNITY

With the development of newer methodologies in more recent years, investigations have explored more comprehensively the impact of probiotics on the broader bacterial community. Before exploring these studies, some recent findings on gut microbial communities, upon which probiotics have potential to exert influence, are highlighted (Table 3). The research by Qin et al[20] contributed greatly to the findings summarized in this Table. Qin et al[20] reported the metagenomic sequencing, assembly, and characterization of 3.3 million nonredundant microbial genes, derived from 576.7 Gb of sequence, from fecal samples of 124 healthy, overweight, obese, and inflammatory bowel disease (IBD) European individuals. A key perspective reinforced by this study is that gut colonization is not simply a mixture of microbes, but an evolved ecosystem that works in concert. Certain types of microbes appeared to be found together, perhaps enabling target functionality (rather than one specific compilation of species) of a healthy gut microbiota.

A few animal and human studies have looked in depth at the impact of probiotics on commensal gut bacterial

TABLE 3. The Gut Microbial Community: Findings to date From Human Microbiome Research Projects [10,20]

The Bacteroidetes and the Firmicutes constitute more than 90% of the known phylogenetic categories and dominate the distal gut microbiota
Prominent clusters include Bacteroidetes and Dorea/Eubacterium/Ruminococcus groups, bifidobacteria, Proteobacteria, and streptococci/lactobacilli groups
Dysbacteriosis is associated with some disease states
No consensus has been reached on what comprises "healthy microbiota"
Studies show substantial diversity of the gut microbiome among healthy individuals, especially infants, but the mature gut microbiome converges to greater between-individual similarity
Although 1000–1150 bacterial species comprise approximately 99% of species isolated from humans, each individual harbors approximately 160 unique species/person
A common bacterial species "core" is shared among at least 50% of individuals
Seventy-five species are common to $>50\%$ of individuals and 57 species are common to $>90\%$ of individuals
Three distinct microbial communities have been identified, irrespective of geographical origin
Much functional similarity exits among bacterial communities from different individuals, even if populations differ
The total number of bacterial genes encoded by colonizing bacteria is approximately 50-fold greater than human genes
536,112 prevalent bacterial genes have been identified, but 2,375,655 bacterial genes are present in fewer than 20%, whereas 294,110 are found in at least 50% of individuals

Almost 40% of the bacterial genes from each individual are shared with at least half of the individuals.

communities. Although the picture is just emerging, some interesting observations have been made.

Using both culture-dependent and culture-independent methods, Veiga et al[21] used a mouse model of IBD to determine the impact of a fermented milk containing *Bifidobacterium animalis* subsp. *lactis* DN-173 010 (*B. lactis*), *Streptococcus thermophilus*, 2 strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactococcus lactis* subsp. *cremoris* on the microbiota. They found that the response to *B. lactis*-containing fermented milk included decreased cecal pH, altered short chain fatty acid profiles (increased acetic acid, propionic acid, and butyric acid and decreased lactic acid) and increased abundance of select lactate-consuming and butyrate-producing bacteria. In addition, scores for intestinal inflammation improved. A particular gut microbiota pattern was associated with response to the probiotic milk, suggesting that microbiota patterns might be useful for identifying responders and nonresponders to probiotic interventions.

Martin et al[22] investigated the impact of feeding a *Lactobacillus paracasei* NCC2461 on gut microbiota, but also on panorganismal parameters. The study compared 4 groups of mice: conventional, conventionalized (germ-free mice removed from germ-free conditions), and 2 groups of human baby microbiota-associated mice. These last mice were inoculated with 7 bacterial strains (*Escherichia coli*, *Bifidobacterium breve, Bifidobacterium longum*, *Staphylococcus epidermidis, Staphylococcus aureus*, *Clostridium perfringens*, and *Bacteroides distasonis*), isolated from stool of a 20-day old healthy infant who was naturally delivered and breast fed. The human baby microbiota mice were divided into 2 groups, one fed *L. paracasei* NCC2461 only and the other fed both NCC2461 and galactooligosaccharide. Microbiological assessments were made on selective culture media. *L. paracasei* supplementation resulted in increased *B. longum*, decreased *S. aureus*, decreased plasma lipoproteins, liver glutamine, and glycogen, and decrease adrenal steroidogenesis. In this model system, *L. paracasei* NCC2461 was shown to change the microbiota to a more favorable profile (reduced *S. aureus* and increased *Bifidobacterium*), but also have broad-reaching metabolic effects.

In human studies, Cox et al[23] tested the impact of a probiotic on the bacterial community structure of 6-month-old infants fed 1×10^9 colony forming units/d of *L. rhamnosus* GG from birth through 6 months of age. Elevated levels of *Lactobacillaceae* and *Bifidobacteriaceae* were observed. Investigators posited that these data are evidence that *L. rhamnosus* GG may function, at least in part, through its ability to promote a stable and functionally redundant commensal bacterial community. This study also found that communities containing high *L. rhamnosus* GG levels clustered and were associated with a distinct bacterial community composition. *L. rhamnosus* GG-rich communities displayed increased "evenness." Such communities were characterized by an even relative abundance of taxa, and not dominated by one or a few species. A community with a high level of evenness is associated with increased ability to resist perturbations. Bacterial communities associated with low *L. rhamnosus* GG levels were characterized by an abundance of species associated with allergic disease development.

Lyra et al[24] demonstrated probiotic-induced changes in fecal microbiota of IBS patients. The probiotic was a blend of 4 strains, *Lactobacillus rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Bifidobacterium breve* Bb99 delivered in a capsule. As this probiotic preparation had been shown in a previous randomized, double-blind, placebo-controlled study to reduce borborygmi in patients with IBS,[15] the gut microbiota from 42 of these study participants were analyzed using quantitative polymerase chain reaction targeted at bacterial phylotypes associated with IBS (not at the whole bacterial community). At 3 and 6 months of probiotic feeding, increases compared with placebo were detected in *Clostridium thermosuccinogenes* and *Ruminococcus torques*, but no changes were observed in 9 other phylotypes assayed. The significance of these microbial population changes was not clear, but the investigators speculated that such modifications may underlie the ability of probiotics to impact IBS symptoms.

Matsumoto et al[25] evaluated the impact of *Lactobacillus casei* strain Shirota-fermented milk on fecal microbiota of healthy individuals with soft stools in a randomized, double-blind, placebo-controlled study. Compared with baseline, total bacterial counts, commensal bifidobacteria populations, and short chain fatty acids (total, acetic acid, propionic acid, and butyric acid) significantly increased. Microbiological and biochemical changes occurred concomitantly with hardening of soft stools.

CONCLUSIONS

Evidence from many lines of research document that probiotics can impact the gut microbiota. The impact can be broad-reaching, as indicated by the studies of Martin et al,[22] which demonstrate microbiota-induced changes organism wide, including reduction in plasma lipoproteins, reduction in liver glutamine and glycogen and reduction in adrenal steroidogenesis.

"*These studies suggest that major mammalian metabolic processes and inter organ cross-talks are strongly connected to the microbial activity, which in turn may have long-term health consequences to the host. In particular, our results further suggest that the gut microbiota should be a nutritional target for future interventions aiming to modulate host carbohydrate and lipid metabolism. Therefore, the integration of both individual metabolic predisposition and latent gut microbial metabolic contribution to the host will provide a future basis to develop optimized nutritional management.*" [22]

Or the effects may be more measured, such as increased level of one or a few bacterial phylotypes. Studies on feeding different *Lactobacillus* probiotics, such as *L. rhamnosus* GG[23] or *L. casei* Shirota[25] have resulted in increases in *Bifidobacterium* populations, demonstrating that probiotics can impact populations of other potentially beneficial commensal bacteria.

Modern studies focused on probiotic-induced microbiota changes are few, making general conclusions from them difficult. However, there is great potential in further conduct of these studies to better elucidate microbiota changes that correlate with probiotic-induced improvements in health or symptom measures. Such changes are likely to be strain-specific and dose-specific, but may lead to identification of commensal bacteria that are causal or correlative biomarkers for beneficial physiological changes.

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