Probiotic-Derived Factors: Probiotaceuticals?1

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Probiotics are broadly defined as living, nonpathogenic microorganisms (usually bacteria) which, when administered in sufficient numbers, exert a positive influence on host health. Mechanisms of probiotic action described to date include adhesion to the intestinal-lumen interface; competition with pathogens for nutrients, receptor binding, and colonization; enhancement of mucosal barrier function; promotion of innate and adaptive immune responses; elaboration of bacteriocins; and modulation of cell kinetics via alterations in the proliferation to apoptosis ratio, with further mechanisms of action gradually becoming elucidated (1). However, it is rare for any individual probiotic to act through a single mechanism, and its biological impact is further influenced by factors including dose, frequency of administration, and the composition of the enteric microflora. Perhaps not surprisingly, given the vast numbers of bacterial species, strains, and substrains in the microflora, the number of potential probiotics, and the complexity of their mechanism(s) of action, is equally diverse.

Although *Escherichia coli* 1917, the first probiotic species to be described, was identified almost a hundred years ago, only recently has there been an upsurge in research into the properties of probiotic-derived factors. Such agents could potentially achieve therapeutic benefit while avoiding risks associated with the administration of live bacteria. It is in this context that Heuvelin et al. (2), in the January issue of this journal, explore the biological properties of factors released by the probiotic, *Bifidobacterium breve* C50. In a 2004 rodent study of experimental inflammatory bowel disease (IBD) utilizing VSL#3, a commercially available combination of 8 probiotic species, Rachmilewitz et al. (3) described protective effects mediated by probiotic DNA. Moreover, live microorganisms were not required to attenuate the colitis, as nonviable probiotics could mediate the antiinflammatory effect.

Probiotic-derived factors have since been described as capable of exerting probiotic activities through each of the previously described mechanisms. However, it is important to distinguish between the concept of probiotic, which is necessarily based on the ingestion of live microorganisms, and the concept of microorganism-derived bioactive compounds that may have useful applications in nutrition and medicine. Bioactive compounds of bacterial or yeast origin, (antibiotics, for example), have been utilized in medicine for decades. Although there are many bacteria-derived products capable of inducing a health benefit, the concept of probiotic is only attributed to microorganisms administered as viable forms, providing the opportunity for a symbiotic relationship between the host, and resident, or in-transit, microorganisms.

Secreted probiotic factors, such as reuterin from *Lactobacillus reuteri*, have been reported to inhibit adhesion and viability of known enteric pathogens, suggesting that probiotic supernatants could be a rich source of new antipathogenic compounds. In an in vitro study in human gastric epithelial cells, spent culture supernatants from certain lactic acid producing bacteria inhibited the growth and attachment of *Helicobacter pylori* (4). Roselli et al. (5) demonstrated that supernatants of *Bifidobacterium animalis* MB5 and *Lactobacillus GG* could inhibit adhesion of *E. coli* K88 to Caco-2 cells, with the supernatant exerting identical beneficial effects following protease digestion, suggesting that proteins were not the active constituent.

In a study of the shrimp pathogen, *Vibrio harveyi*, supernatants from 3 Bacillus species (*B. subtilis, B. licheniformis*, and *B. megaterium*) inhibited *V. harveyi* growth and suppressed hemolytic activity (6). The cell-free supernatants produced by *Bacillus probiotics* may have inhibited Vibrio disease by modulating Vibrio cell-to-cell communications, suggesting potential for Bacillus supernatants to form the basis of a new treatment approach, or preventative modality, for Vibrio-associated diseases afflicting humans, such as cholera.

Probiotic supernatants capable of depressing immune responses could be advantageous in IBD, and, indeed, effects on the mucosal immune response and inflammation have been ascribed to probiotic-derived factors in this condition. For example, *Lactobacillus fermentum* BR11 has recently been demonstrated to exhibit probiotic properties in a rat model of colitis. Frick et al. (7) investigated *L. fermentum* supernatant for its capacity to inhibit the proinflammatory responses of HeLa 229 cells to *Yersinia enterocolitica* infection. Treatment with *L. fermentum* supernatant inhibited interleukin-8 secretion and decreased nuclear factor kappa B activation following infection. Effectiveness of the *L. fermentum* supernatant was diminished upon treatment with phospholipase C, indicating a key role for a secreted phospholipid in the antiinflammatory effect. Considered together, future development of probiotic supernatants should ideally include a repertoire of screening assays targeted at elucidating effects on the different components of the systemic and mucosal immune response.
Certain probiotic species exert their beneficial effects through an improvement of intestinal barrier function, with reduced permeability to pathogens, mediated by modulation of the biofilm, mucus layer, and/or tight junctions. The supernatant of VSL#3 has been demonstrated to improve epithelial barrier function, attributed to products secreted by B. infantis (8). B. infantis conditioned media increased trans-epithelial resistance in T84 cells, in addition to expression of tight-junction specific proteins zonula occludens 1, occluding, and claudin-2. Wildtype and interleukin-10 deficient (IL-10−/−) mice treated orally with B. infantis supernatant exhibited a decrease in colonic permeability, an attenuation of colonic inflammation, and a decrease in interferon-gamma secretion (8). Cell-free supernatants from B. lactis 420 have also been shown to protect tight junctions when administered prior to the cell-free supernatant of E. coli O157:H7 (9). Determining the effects of newly developed probiotic supernatants on intestinal permeability would be a logical approach to identify probiotic-derived compounds capable of improving barrier function.

Arguably, some of the new mechanisms of action attributed to specific probiotics, and their supernatants, could hold the greatest therapeutic potential. In the January issue, Heuvelin et al. (2) describe an alleviation of chloride secretion by the HT-29–19A human intestinal epithelial cell line, induced by administration of conditioned medium from the probiotic, Bifidobacterium breve C50. Exacerbated chloride secretion resulting from pathogenic bacteria, bacterial toxins, or neuroimmune activation can result in the clinical manifestation of severe diarrhea. Although an in vitro study using Ussing chambers, the finding that soluble factors from B. breve C50 could significantly downregulate chloride secretion induced by carbachol and forskolin, suggests important implications for the control of water balance and diarrhea. Indeed, the chloride-regulatory properties of soluble factors from B. breve C50 could have implications for the symptomatic control of other conditions characterized by altered chloride secretion, such as cystic fibrosis. Moreover, the recent discovery that supernatants from E. coli Nissle 1917 could modulate colonic motility in an organ bath system suggests applications for probiotic-conditioned media in the alleviation of fecal incontinence and other conditions with impaired gut contractility (10).

The potential utility of probiotic-derived factors in cancer therapy represents an intriguing new frontier. Fatty acids such as butyrate have demonstrated anticarcinogenic properties, primarily acting through a pro-apoptotic mechanism. Clostridium butyricum produces high levels of butyrate. Similarly, Lactobacillus acidophilus La-5 releases conjugated linoleic acid, a potent anticarcinogenic agent (11). Moreover, extracellular bioactive compounds from Lactobacillus and Bifidobacterium strains have demonstrated antimutagenic properties against benzo[a]pyrene and sodium azide (12); and supernatants from S. thermophilus TH-4 (13) resulted in decreased crypt fission, suggesting therapeutic utility in disorders characterized by increased crypt fission, such as colorectal carcinoma. Although investigations into the effects of probiotic supernatants on the processes of transformation and neoplasia are in their infancy, this could represent a promising new direction in cancer treatment.

Developing and implementing reproducible assay systems to quantify the biological properties of probiotic supernatants will be challenging. Moreover, determining the precise composition of secreted products from probiotic bacteria is a daunting prospect, and it will undoubtedly vary dependent on species, strain, microenvironment, and culture conditions. Although it is perhaps too early to predict the development of a “probiotica-ceutical” industry, undeniably, probiotic-derived factors are furthering our understanding of probiotic mechanisms.

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Literature Cited