

## Human gut microbiota and its relationship to health and disease

Taylor C Wallace, Francisco Guarner, Karen Madsen, Michael D Cabana, Glenn Gibson, Eric Hentges, and Mary Ellen Sanders

*Probiotics are live microorganisms that confer a health benefit on the host when administered in appropriate amounts. Over 700 randomized, controlled, human studies have been conducted with probiotics thus far, with the results providing strong support for the use of probiotics in the clinical prevention or treatment of gastrointestinal tract disorders and metabolic syndrome. The present review is based on webinar presentations that were developed by the American Gastroenterological Association (AGA) in partnership with the International Scientific Association for Probiotics and Prebiotics (ISAPP) and the North American branch of the International Life Sciences Institute (ILSI North America). The presentations provided gastroenterologists and researchers with fundamental and current scientific information on the influence of gut microbiota on human health and disease, as well as clinical intervention strategies and practical guidelines for the use of probiotics and prebiotics.*

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### INTRODUCTION

Louis Pasteur is best remembered for his remarkable breakthroughs in demonstrating the germ theory of disease, which proposes that microorganisms that infect animals and humans cause infectious diseases, as well as for developing strategies, such as vaccines and pasteurization, to prevent and combat these diseases. In 1885, he reported the following:

For several years during discussions with young scientists in my laboratory, I have spoken of the interest in feeding from birth a young animal (rabbit, guinea pig, dog or chicken) with pure nutritive products which have been artificially and totally deprived of the common micro-organisms. Without affirming anything, I do not conceal the fact that I would undertake such a study with the preconceived idea that under these conditions life would become

impossible. If this work could be developed simply, one could then consider the study of digestion by the systematic addition to the pure food of one or more well defined micro-organisms.<sup>1</sup>

With his awareness of the causative role of some specific microbes in producing disease, the prominent French scientist presumed that other microbes would be essential for life. Pasteur suggested that animals would not be able to survive when totally deprived of “common microorganisms,” and he predicted the potential use of microorganisms in foods in order to improve digestive functions.

Bernard S. Wostmann of the Lobund Laboratory at the University of Notre Dame in Indianapolis, Indiana, quoted Pasteur’s words a century later.<sup>2</sup> Researchers from the Lobund Laboratory fully developed appropriate facilities and technologies to breed experimental animals under germ-free conditions and to study the impact of

Affiliations: *TC Wallace* and *E Hentges* are with ILSI North America, Washington DC, USA. *F Guarner* is with the Digestive System Research Unit, University Hospital Vall d’ Hebron, Barcelona, Spain. *K Madsen* is with the Department of Medicine, Division of Gastroenterology, University of Alberta, Edmonton, Alberta, Canada. *M Cabana* is with the University of California San Francisco Benioff Children’s Hospital, San Francisco, California, USA. *G Gibson* is with the Department of Food and Nutritional Sciences, The University of Reading, Reading, Berkshire, United Kingdom. *ME Sanders* is with the International Scientific Association for Probiotics and Prebiotics, Davis, California, USA.

Correspondence: *TC Wallace*, ILSI North America, 1156 15th Street, NW, Suite 200, Washington, DC 20005 USA. E-mail: taylor.wallace@me.com, Phone: +1-202-659-0074; Fax: +1-202-659-3859.

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microbial colonization on host physiology. The work of Wostmann and his colleagues demonstrated that, contrary to Pasteur's presumption, animal life (as shown with mammals and birds) is possible in the absence of microbial colonization. However, a major challenge to achieving survival in the germ-free state was to develop adequate diets to meet the extraordinary nutritional requirements in the absence of microbial colonization.<sup>2</sup> Such germ-free animals have very high nutritional requirements in terms of food composition and quantity, and (in addition to other possible factors) do not develop normally in terms of body anatomy and physiology. Microbial colonization of animals is not essential for life, but it is critical for normal growth and development.

Bacteria have reportedly been on Earth for 3.5 billion years, appearing approximately 1 billion years after the Earth's crust was formed.<sup>3</sup> Early microbial communities synthesized hydrocarbonated compounds and were capable of both photosynthetic oxygen production and respiratory oxygen consumption. Free oxygen in the atmosphere has been widely assumed to originate from the presence of morphologically cyanobacteria-like fossils in Earth's early history, suggesting that oxygenic photosynthesis and aerobic respiration are processes derived from microbial biochemistry.<sup>3</sup> Microbes continue to be ubiquitous and vital worldwide. Their diverse contributions affect every aspect of life, from human infections, to the treatment of chemical contamination, to the cycling of the most critical elements for maintaining life. Evolution, disease, corrosion, degradation, bioremediation, and global cycling are a few of the many thousands of ways in which the impact of microbial communities is felt.<sup>4</sup> Taking the global impact of microbes into consideration, Pasteur's presumption was not inaccurate.

The present review focuses on the beneficial effects of microorganisms for the promotion of human health. As predicted by Pasteur,<sup>1</sup> the inclusion of specific microorganisms in food may contribute to the improvement of bodily functions. Scientific research has identified specific strains of live microorganisms called probiotics, which can induce health benefits on the host when administered in adequate amounts. In addition, scientists have developed the concept of food that can be consumed in order to selectively promote the growth and activity of beneficial bacteria that colonize the intestinal tract. These foods are called prebiotics. A considerable number of scientific publications have reported interesting observations in basic science, as well as in applied human studies. As of August 2010, the PubMed database included approximately 9,000 articles on probiotics or prebiotics. The aim of the current review was to summarize the evidence accumulated during the past decade, as presented in the 2010 webinars organized by the American Gastroentero-

logical Association (AGA) in partnership with the International Scientific Association for Probiotics and Prebiotics (ISAPP) and the North American branch of the International Life Sciences Institute.

## GUT MICROBIOTA AND HEALTH

The human gastrointestinal (GI) tract houses over  $10^{14}$  microbial cells with over 1,000 diverse bacterial types, mostly in the colon. The GI tract is a sterile environment at birth, and bacterial colonization begins during the delivery process (from the maternal fecal or vaginal flora and/or the environment). The bacteria that colonize the large gut initially are facultative anaerobic strains such as *Escherichia coli* and *Streptococcus* spp. These first colonizers metabolize any traces of oxygen in the gut, thereby reducing the environment into one with strong anaerobic conditions. The subsequent colonizing bacteria are largely determined by the feeding profile of the infant. Human milk, apart from being a nutritious, complete food for infants, also induces marked changes in probiotic levels in the infant gut. Factors including microbiota of the female genital tract, sanitary conditions, obstetric techniques, vaginal or caesarean mode of delivery, and type of feeding have an immediate effect on the level and frequency at which various species colonize the infant gut. The final phase of microbiota acquisition occurs at weaning, when a complex microflora develops. The majority of bacteria in the adult gut are non-spore-forming anaerobes, the most numerically predominant of which include *Bacteroides* spp. and *Bifidobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Lactobacillus* spp., *Fusobacterium* spp., and various gram-positive cocci. Bacteria that are present in lower numbers include *Enterococcus* spp., *Enterobacteriaceae*, methanogens, and dissimilatory sulphate-reducing bacteria.

Colonic microorganisms are readily able to degrade available substrates. These may be derived from either the diet or endogenous secretions. Major substrates available for colonic fermentation are starches and soluble dietary fibers. Other carbohydrate sources available for fermentation in lower concentrations essentially include oligosaccharides and portions of non-absorbable sugars and sugar alcohols. In addition, proteins and amino acids can be effective growth substrates for colonic bacteria, whereas bacterial secretions, lysis products, sloughed epithelial cells, and mucins may also contribute. A wide range of bacterial enzymes degrade these materials. Intestinal bacteria are then able to ferment these intermediates to organic acids, histamine, carbon dioxide, and other neutral, acidic, and basic end products. Fermentation by gut bacteria consists of a series of energy-yielding reactions that do not use oxygen in the respiratory chains.

Because of their metabolic activities and fermentation end products, gut bacteria can be categorized as either beneficial or potentially pathogenic. Health-promoting effects of the microbiota may include the following: immunostimulation, improved digestion and absorption, vitamin synthesis, inhibition of the growth of potential pathogens, cholesterol reduction, and lowering of gas distension. Harmful effects include carcinogen production, intestinal putrefaction, toxin production, diarrhea/constipation, liver damage, and intestinal infections. Bifidobacteria and lactobacilli are considered examples of health-promoting constituents of the microbiota. They may aid lactose digestion in lactose-intolerant individuals, reduce constipation and infantile diarrhea, assist resistance to infections, and reduce inflammatory conditions in the gut. Both probiotics and prebiotics can fortify the lactate-producing microbes of the human or animal gut.<sup>5,6</sup> The probiotic approach advocates the use of living organisms in the diet. An alternative approach aimed at increasing the amount of health-promoting bacteria in the gut has also been investigated, whereby these bacteria are selectively promoted by the intake of certain non-digestible carbohydrates known as prebiotics.

A good probiotic has several desirable characteristics. For example, it exerts a beneficial effect on the consumer, is nonpathogenic and nontoxic, contains an efficacious number of viable cells, has the capacity to survive and metabolize in the gut, retains viability during storage and use, and should have good sensory qualities if incorporated into a food.

A dietary prebiotic is a food ingredient refractory to the human digestive process that is selectively fermented by the gut microbiota, resulting in specific changes in the composition and/or activity of the gastrointestinal microbiota and thus conferring benefit(s) upon host health. There are three required criteria for a prebiotic effect: 1) resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption; 2) fermentation by intestinal microbiota; and 3) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being.<sup>7</sup>

Increased evidence from over 700 randomized, controlled, human studies provides strong evidence that select gut microbiota may aid in preventing or treating various GI tract disorders, promoting GI health, and preventing metabolic syndrome. Gut microbes are known to be involved in many clinical states; however, their precise role is not clear and additional research is needed to determine if these microbes have a causal or associative relationship. Factors such as pH of the gut contents, nutrient availability, redox potential within the tissue, age of the host, host health, bacterial adhesion, bacterial cooperation, mucin secretions containing immunoglobulins, bacterial antagonism, and transit time may affect the

diversity and quantity of microbiota present in the various regions of the GI tract.<sup>8</sup> Acidification of the intestinal environment by probiotics may inhibit the growth of pathogens and the production of toxic compounds such as ammonia and amines. In a diverse microbial population, carbohydrate fermentation yields short-chain fatty acids<sup>9</sup> such as butyrate, which has been known to inhibit DNA synthesis and stimulate apoptosis. These compounds may play a significant role in the prevention of cancer of the GI tract. Carbohydrate fermentation and short-chain fatty acid production significantly improve the absorption of calcium, magnesium, and phosphorus.<sup>9</sup> Several members of the intestinal microbiota produce vitamins and minerals and provide them to the host. Germ-free animals require 30% more energy in their diet, and supplementation with vitamins K and B is mandatory to maintain their body weight.<sup>9</sup>

Various levels of host-microbe interaction can be distinguished, including microbe-gut epithelium interaction, microbe-immune system interaction, and microbe-microbe interaction. Bifidobacteria have been shown to modulate the immune system, produce digestive enzymes, and restore activities of the gut microbiota following antibiotic therapy.<sup>10</sup> The gut microbiota is reported to contribute to human protein homeostasis. Germ-free animals are highly susceptible to infections, providing evidence that the intestinal microbiota is considered an important defense barrier. Probiotics can compete for some of the same attachment sites as pathogens, use the same nutrients, and produce antimicrobial compounds that inhibit the growth of pathogens.<sup>11,12</sup> Studies have demonstrated that the immune system is influenced by the gut microbiota, which provides a stimulus for its development. The immune system is immature at birth and develops upon exposure to the gut microbiota. The innate immune system allows the host to sense a concrete microbial environment in order to promote the release of signaling molecules (cytokines and chemokines), thereby initiating an immune response.<sup>13</sup> Adhesion of gut microbes and pathogens shows great variability among strains and does not guarantee persistence; however, many probiotics are known to inhibit adhesion and displace pathogens such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium difficile*.<sup>14</sup>

Many studies have confirmed the efficacy of the gut microbiota for treating many disorders of varying severity and modulating the colonic microbiota toward a healthier composition. Certain limitations, including nutrient availability and the ability of probiotics to survive the host's physiochemical protective barriers in order to reach the lower GI tract, must be overcome before an ecological niche can become established. If this can be accomplished, the gut microbiota may have a

number of remarkable postulated health effects on acute and chronic disease in humans.

## PROBIOTIC INTERVENTIONS TO INFLUENCE HEALTH AND DISEASE

Probiotics are currently the subject of significant clinical research. A growing body of work now exists describing the role of various probiotic strains in ameliorating chronic intestinal inflammation, diarrhea, constipation, vaginitis, irritable bowel syndrome, atopic dermatitis, sepsis, food allergies, and liver disease. Substantial evidence has shown that probiotics can modulate systemic and mucosal immune function, improve intestinal barrier function, alter gut micro-ecology, and exert metabolic effects on the host. It is important to reiterate, however, that it is not possible to generalize these individual effects for every probiotic, and each individual strain must be tested for each property. The key to the effective use of probiotics in treating human disease is to match the correct probiotic strain with the desired clinical outcome. Table 1 summarizes the findings of recent studies published in 2009–2010 on the effects of probiotics on systematic and mucosal immune function, barrier function, and metabolism (divided into human clinical trials, in vitro human cell studies, and animal models).<sup>15–42</sup>

### Effects on systemic and mucosal immune function

Functions of the immune system at both a systemic level and a mucosal level can be modulated by live bacteria and by bacterial components in the intestine. Gut epithelial and immune cells are continually sampling gut microbes,<sup>43</sup> and bacterial strains can signal through pattern-recognition receptors, resulting in the modulation of various intracellular signaling pathways.<sup>44</sup> The active signaling components of bacteria include the following: enzymes, secreted factors, surface-layer proteins, isolated DNA, bacterial formulated peptides such as N-formyl-methionyl-leucyl-phenylalanine (FMLP), lipopolysaccharide (LPS), and peptidoglycan cell wall constituents that signal through Toll-like receptors (TLRs). TLRs are a family of innate immune receptors that detect multiple microbe-associated molecular patterns, including LPS by TLR4, lipoproteins and lipoteichoic acids by TLR2, double-stranded RNA by TLR3, single-stranded RNA by TLR7, flagellin by TLR5, and CpG DNA by TLR9.<sup>45</sup> Recent studies have identified a key role for TLR2 signaling in the maintenance of barrier function and stimulation of host defense mechanisms.<sup>46</sup>

Intestinal epithelial cells play an active role in innate immune responses by releasing both chemokines and cytokines that modulate underlying dendritic cell and macrophage responses.<sup>44</sup> Probiotics modulate the nuclear

factor-kappa B (NF- $\kappa$ B) signal transduction pathway in epithelial cells through TLRs and via the release of soluble mediators, resulting in inhibition of NF- $\kappa$ B translocation to the nucleus and reduction in the degradation of I $\kappa$ B kinase through modulation of proteasome function.<sup>47,48</sup> Proteasomes play a key role in the degradation of endogenous and exogenous proteins for antigen presentation by both major histocompatibility complex class I and II molecules.<sup>49</sup> A therapeutic role for proteasome inhibitors has been documented for several inflammatory disorders, including psoriasis, rheumatoid arthritis, asthma, sepsis, and inflammatory bowel disease.<sup>50,51</sup> Probiotics inhibit the degradation of proteasomes and I $\kappa$ B through both a TLR9-mediated mechanism<sup>52</sup> and via the release of soluble mediators.<sup>53</sup>

Apart from their effects on intestinal epithelial cells, probiotics have also been shown to alter mucosal immune function in a strain-dependent fashion in a number of other ways, including the enhancement of antibody production,<sup>54–56</sup> the increase of phagocyte and natural killer cell activity,<sup>54</sup> and the induction of regulatory dendritic cells and CD4+Foxp3+T cells.<sup>57</sup> Some probiotic bacteria, particularly bifidobacteria, induce a pattern of maturation of dendritic cells characterized by the release of small amounts of tumor necrosis factor- $\alpha$  and interleukin (IL)-12, with increased levels of IL-10.<sup>58</sup> This increased IL-10 production may then have a direct anti-inflammatory effect and may also induce the generation of regulatory T cells. In contrast to the effects of bifidobacteria, some lactobacilli generate a dendritic cell phenotype with increased costimulatory marker expression but low production of proinflammatory cytokines.<sup>59</sup> Overall, probiotic bacteria tend to induce an immunoregulatory phenotype in dendritic cells, rather than an aggressive immune response.

### Probiotics and barrier function

Probiotic bacteria enhance epithelial barrier function through several mechanisms, including effects on epithelial tight junction proteins, increased production of intestinal mucus, enhanced mucosal immunoglobulin A responses, induction of cellular heat-shock proteins, prevention of epithelial apoptosis, and increased stimulation of defensin production. Oral administration of the probiotic mixture VSL#3 (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Streptococcus salivarius* subspecies *thermophilus*) has been shown to normalize barrier function in animal models of colitis,<sup>48</sup> and bioactive factors released from *B. infantis* were shown to enhance resistance both in an animal model and in cell culture models.<sup>60</sup> Studies have

**Table 1 Summary of recent studies (2009–2010) on the effects of probiotics on systemic and mucosal immune function, barrier function, and metabolism.**

| Type  | Organism  | Model system   | Findings   | Reference                                     |
|---|---|--|--|---|
| Human clinical trials   | <i>Lactobacillus delbrueckii bulgaricus</i> in yogurt   | Healthy elderly  | Increased NK cell activity   | Ndagijimana et al. (2009) <sup>15</sup>       |
|   | <i>Lactobacillus salivarius</i> CECT5713  | Phase 2 randomized, double-blind, placebo-controlled in 40 healthy adults  | Reduced risk of common cold<br>Increased frequency of defecation<br>Increased % NK cells and monocytes<br>Increased plasma IgM, IgA, IgG<br>Increased plasma IL-10 | Sierra et al. (2010) <sup>16</sup>            |
|   | <i>Streptococcus thermophilus</i>   | Double-blind, placebo-controlled trial in 162 children of low socioeconomic status for 4 months                                | No difference in response to vaccination   | Perez et al. (2010) <sup>17</sup>             |
|   | <i>Lactobacillus casei</i>  | Double-blind, randomized, placebo-controlled trial in 44 allergic children for 3 months  | No difference in days of fever or number of infections<br>Decreased plasma IgE and increased Treg  | Martinez-Canavate et al. (2009) <sup>18</sup> |
|   | <i>Lactobacillus gasseri</i> CECT5714<br><i>Lactobacillus coryniformis</i> CECT5711 in yogurt |  | Increased gut sIgA<br>Increased NK cells   |   |
|   | <i>Bacillus coagulans</i> GBI-30  | 10 healthy adults treated for 30 days, then exposed to adenovirus and influenza A  | No difference in eosinophils, basophiles<br>Probiotic treatment increased T cell production of TNF- $\alpha$ in response to virus exposure                         | Baron (2009) <sup>19</sup>                    |
|   | <i>Lactobacillus</i> F19  | Double-blind, placebo-controlled randomized trial in 179 infants from 4–13 months of age                                       | Reduced incidence of eczema in probiotic group<br>Increased IFN $\gamma$ /IL-4 mRNA ratio in probiotic group   | West et al. (2009) <sup>20</sup>              |
|   | <i>Bifidobacterium lactis</i> and long-chain fatty acids                                      | Randomized, double-blind, controlled, parallel-group trial in 142 healthy infants for 7 months                                 | No difference in growth between groups<br>No difference in response to vaccines  | Gibson et al. (2009) <sup>21</sup>            |
|   | Symbioflor 2 – <i>Escherichia coli</i>  | Administered to 23 healthy adults for 3 weeks  | Increased fecal beta-defensin2 in 78%  | Mondel et al. (2009) <sup>22</sup>            |
|   | <i>Lactobacillus sakei</i>  | Double-blind, placebo-controlled trial in 88 children with atopic eczema-dermatitis syndrome for 12 weeks                      | Probiotic group had decreased chemokine levels and clinical improvement  | Woo et al. (2010) <sup>23</sup>               |
| VSL3  | Ulcerative colitis patients   | Treatment of UC patients with probiotic increased regulatory cytokines and lowered pro-inflammatory cytokine secretion from DC | Ng et al. (2010) <sup>24</sup>   |   |
| <i>Bifidobacterium bifidum</i> BGN4,<br><i>B. lactis</i> ADO11, <i>L. acidophilus</i> AD031<br><i>Lactobacillus acidophilus</i> ,<br><i>Bifidobacterium longum</i> ,<br>fructooligosaccharides<br><i>B. longum</i> , psyllium | 112 pregnant women treated from 4–8 weeks before delivery and until infants were 6 months old | Probiotic decreased prevalence of eczema 1 year<br>No difference in serum total IgE  | Kim et al. (2010) <sup>25</sup>  |   |
|   | 16 healthy subjects studied after 1 month of treatment  | Metabolic profiles in feces assessed by nuclear magnetic resonance   | Ndagijimana et al. (2009) <sup>15</sup>  |   |
|   | 120 ulcerative colitis patients treated for 4 weeks   | Synbiotic therapy increased quality of life  | Fujimori et al. (2010) <sup>26</sup>   |   |

|                          |   |   |   |   |
|--------------------------|---|---|---|---|
| Human cells and in vitro | <i>B. bifidum</i>   | Human eosinophils<br>PBMC   | <i>B. bifidum</i> inhibited <i>Clostridium difficile</i> -induced release of neurotoxin<br>Increased NK cell activity<br>Increased proinflammatory and anti-inflammatory cytokines<br>Induced DC maturation<br>Effects on cytokine production dependent upon strain   | Hosoki et al. (2010) <sup>27</sup><br>Dong et al. (2009) <sup>28</sup><br>Lopez et al. (2010) <sup>29</sup>   |
|                          | <i>B. longum</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium bifidum</i> , <i>Bifidobacterium animalis</i><br>LGG, <i>B. breve</i>              | Human monocyte-derived dendritic cells  |   | Van Hoffen et al. (2010) <sup>30</sup>  |
| Animal models            | <i>L. casei rhamnosus</i>   | PBMC/HT-29 cultured cells<br>PBMC/human monocyte cell line (THP-1)  | UV-killed and BB increased Th1 and/or Treg response from PBMC<br>LGG enhanced Th1 response from HT-29 cells<br>Heat-stable molecules induced immune cell apoptosis through a mitochondrial pathway<br>Inhibited LPS-induced inflammatory cytokines<br>Increased human beta-defensin 2 expression and secretion<br>Inhibited LPS-induced IL-23 secretion<br>Live <i>L. casei</i> decreased secretion of TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-6, IL-8, and CXCL-1 | Chiu et al. (2010) <sup>31</sup><br>Paolillo et al. (2009) <sup>32</sup><br>Lopis et al. (2009) <sup>33</sup> |
|                          | <i>Lactobacillus plantarum</i>  | Caco-2 cells  | Live <i>L. casei</i> inhibited cytokine secretion in response to <i>E. coli</i><br>Probiotic prevented unconjugated bilirubin-induced decrease in epithelial resistance   | Zhou et al. (2010) <sup>34</sup>  |
|                          | <i>E. coli</i> ATCC 35345, <i>L. casei</i> DN-114 001   | ileal mucosal explants from patients with Crohn's disease   | Probiotic restored PKC activity and tight junction protein levels<br><i>L. rhamnosus</i> prevented TNF- $\alpha$ -induced decrease in epithelial resistance   | Miyauchi et al. (2009) <sup>35</sup>  |
|                          | <i>L. plantarum</i>   | Caco-2 cells  | Increased IL-8 secretion<br>Association of <i>Lactobacillus</i> or <i>Bifidobacterium</i> with sIgA increased adhesion and increased transepithelial resistance   | Mathias et al. (2010) <sup>36</sup>   |
|                          | <i>Lactobacillus rhamnosus</i>  | Caco-2/T84 cells  | Increased phosphorylation of ZO-1 and occludin<br>Increased TSLP production<br>Exopolysaccharides isolated from <i>L. acidophilus</i> activated autophagic cell death by induction of beclin-1 and GRP78  | Kim et al. (2010) <sup>37</sup>   |
|                          | <i>Lactobacillus acidophilus</i> 606  | HT-29 cells   | Increased villus height and width in duodenum   | Im et al. (2009) <sup>38</sup><br>Chon and Choi (2010) <sup>39</sup><br>Yang et al. (2009) <sup>40</sup>      |
|                          | <i>Bacillus polyfermenticus</i><br><i>L. plantarum</i><br><i>Bifidobacterium adolescentis</i> BBMN23,<br><i>B. longum</i> BBMN68<br><i>L. rhamnosus</i> | HIMEC<br>Murine macrophage cell line (RAW264)<br>Balb/c mice for 4 weeks  | Increased angiogenesis in a NF- $\kappa$ B/IL-8-dependent manner<br>Increased IL-1 $\beta$ , IL-6, TNF- $\alpha$  | Mathias et al. (2009) <sup>35</sup><br>Martin et al. (2009) <sup>41</sup>                                     |
|                          | <i>Lactobacillus paracasei</i> , prebiotics<br><i>Bacillus polyfermenticus</i><br><i>E. coli</i> Nissle 1917  | DSS-induced colitis<br>Conventional, and humanized gnotobiotic mice inoculated with human baby microbiota<br>Murine colitis<br>Murine model of mite allergy | Promoted angiogenesis in mucosa during recovery of colitis<br>Probiotic inhibited the development of airway eosinophilia and neutrophilia through TLR4 signalling   | Chon and Choi (2009) <sup>38</sup><br>Adam et al. (2010) <sup>42</sup>  |

Abbreviations: DC, dendritic cell; DSS, dextran sulfate sodium; HIMEC, human intestinal microvascular endothelial cells; MLCK, myosin light chain kinase; NK, natural killer; PBMC, peripheral blood mononuclear cells; PKC, protein kinase C; sIgA, secretory IgA; TSLP, thymic stromal lymphopoietin; ZO-1, zonulin occludin 1.

shown that *S. salivarius* subspecies *thermophilus* and *L. acidophilus* enhance phosphorylation of actinin and occludin in the tight junction region of epithelial cells, thereby inhibiting the invasion of pathogens into human intestinal epithelial cell lines.<sup>61</sup> Several strains of lactobacilli upregulate mucus production by stimulating mucin gene and protein expression.<sup>62,63</sup> Another mechanism by which probiotics can enhance gut barrier function is via enhanced production of cytoprotective molecules. Heat-shock proteins are constitutively expressed in epithelial cells and are induced in cells by stress in order to help maintain homeostasis.<sup>64</sup> Soluble factors released from *Lactobacillus* GG induce cytoprotective heat-shock protein synthesis in intestinal epithelial cells in a manner that is dependent on the p38 kinase and c-Jun N-terminal kinase mitogen-activated protein kinase.<sup>65</sup> Quorum-sensing molecules secreted by *Bacillus subtilis* also induce epithelial expression of cytoprotective heat-shock proteins.<sup>66</sup> Probiotics can prevent cytokine- and oxidant-induced epithelial damage by promoting cell survival (noting the imbalance between cell survival and apoptosis that occurs in inflammatory bowel disease). Studies have shown that *Lactobacillus* GG and soluble factors (p75 and p40) released from this strain prevent epithelial cell apoptosis through activation of anti-apoptotic Akt in a phosphatidylinositol-3'-kinase-dependent manner and by inhibiting activation of the pro-apoptotic p38/mitogen-activated protein kinase.<sup>67,68</sup> Finally, some probiotics stimulate release of defensins from epithelial and Paneth cells. Researchers have shown that *Lactobacillus fermentum* and *E. coli* Nissle 1917 both stimulate  $\beta$ -defensin mRNA and the protein secretion manner through regulation of the NF- $\kappa$ B- and AP-1-dependent pathways.<sup>69,70</sup>

### Antimicrobial and metabolic actions

Probiotics suppress the growth and invasion of pathogens by numerous mechanisms, including competitively excluding pathogens and breaking down undigested polysaccharides to produce short-chain fatty acids, thus reducing pH and inhibiting pathogen growth. Several strains of *Lactobacillus* and *Bifidobacterium* are able to compete with pathogens for binding to intestinal epithelial cells; they are also able to displace pathogens even if the pathogens have already attached.<sup>71-74</sup> Probiotic inhibition of pathogen adherence to epithelial cells is mediated partially by competition for lectin binding sites on glycoconjugate receptors on the brush border membrane surface.<sup>75,76</sup> Recent studies of the metabolic effects of oral administration of *Lactobacillus paracasei* and *Lactobacillus rhamnosus* in humanized microbial genome mice (germ-free mice colonized with a human infant microbiota) have demonstrated that probiotics modify the gene

expression of resident microbes. In addition, probiotics were shown to alter hepatic lipid metabolism in the host, lower plasma lipoprotein levels, and stimulate glycolysis.<sup>77</sup> Other studies using whole-genome transcriptional profiling of individual bacterial species have demonstrated that orally administered probiotics alter the gene expression of resident gut microbes. In these studies, *B. longum* caused an expansion of the diversity of polysaccharides targeted for degradation by *Bacteroides thetaiotaomicron*.<sup>78</sup> Interestingly, the overall effects on these microbial genomes were dependent on the genetic background of the host mouse.<sup>78</sup> Taken together, these types of system analyses demonstrate the far-reaching mucosal and systemic effects of oral probiotic consumption; in the future, they will undoubtedly lead to a much clearer understanding of the mechanistic basis of probiotic actions.

### PRACTICAL GUIDELINES FOR THE USE OF PROBIOTICS AND PREBIOTICS

Although the term “probiotic” was not coined until 1953, the healthful effects of certain bacteria have been noted for over a century.<sup>79</sup> In 1906, for example, Tissier noted that significant stool colonization with bifidobacteria was associated with decreased likelihood of diarrhea in children.<sup>80</sup> In the last 25 years, there has been increasing research, development, and application of probiotic supplements for different indications, such as antibiotic-associated diarrhea, necrotizing enterocolitis, inflammatory bowel disease, and extraintestinal disorders including atopic dermatitis and recurrent urinary tract infections.<sup>81</sup> As these applications of probiotics are increasingly considered for therapeutic use, general practical considerations arise.

The Food and Agriculture Organization of the United Nations and the World Health Organization define probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” There are some clinical implications for this definition: e.g., probiotics are not limited to just bacteria; certain strains of yeast can be used as a probiotic.<sup>82</sup> In addition, the presence of “live cultures” does not necessarily mean that the product is probiotic. The probiotic strain(s) must be present in adequate amounts and be shown to confer a health benefit.

Prebiotics are not probiotics; although the names sound the same, they are vastly different. By definition, a prebiotic is a food ingredient that is nondigestible by the host and has a beneficial effect through its selective metabolism in the GI tract.<sup>7</sup> Examples of prebiotics include inulin, fructooligosaccharides, and galactooligosaccharides. Human breast milk contains a significant amount (5–8 g/L) of unique oligosaccharides, which are

**Table 2 Dose studies and outcomes of probiotics in clinical trials.**

| Dose (CFU/day)       | Strain                                    | Duration | Effect   | Reference                              |
|----------------------|---|----------|--|--|
| $2.0 \times 10^7$    | <i>Bifidobacterium longum</i> (BB536)     | 16 weeks | Japanese cedar allergy   | Xiao et al. (2006) <sup>85</sup>       |
| $1.0 \times 10^8$    | <i>Lactobacillus reuteri</i> (ATCC 55730) | 3 weeks  | Decrease <i>Streptococcus mutans</i> associated with dental caries | Caglar et al. (2006) <sup>86</sup>     |
| $1.0 \times 10^{10}$ | <i>Lactobacillus</i> GG                   | 24 weeks | Prevention of atopic dermatitis                                    | Kalliomäki et al. (2003) <sup>88</sup> |
| $3.6 \times 10^{12}$ | VSL#3                                     | 4 weeks  | Pouchitis  | Gionchetti et al. (2007) <sup>88</sup> |

Abbreviation: CFU, colony-forming unit.

similar to fructooligosaccharides.<sup>83</sup> Synbiotics are defined as a combination of probiotics and prebiotics.

### Dosage and administration: issues to consider

Supplements can theoretically provide a more consistent and relatively higher dose of probiotics with a much lower ingested volume than food products. In addition, food products require adequate amounts of probiotic strains in order to confer a requisite health benefit. Once again, it is important to note that the mere presence of live cultures in a food does not necessarily mean the product is probiotic. Food products can, however, offer the additional benefit of other nutritional components and/or prebiotics.

When evaluating the current literature, it is important that clinicians pay close attention to the strain (not just the genus and species) being used in a particular study, because the efficacy of one probiotic strain does not imply that other strains will be equally efficacious. The American Type Culture Collection (ATCC) repository, established in 1914 and incorporated in 1925, contains over 18,000 bacterial strains, including 4,600 species from 963 genera. Different probiotic strains exert their beneficial effects via a variety of different mechanisms<sup>84</sup> and may be synergistic with other microbiota. One strain of a probiotic may have a different set of properties and clinical effects than another strain of probiotic, even if they are the same genus and species.

Studies to date have used doses ranging from  $2 \times 10^7$  colony-forming units (CFU) per day to  $3.2 \times 10^{12}$  CFU per day (Table 2).<sup>85–88</sup> There are no uniform dosing recommendations for probiotics at this time, and frequency can range from twice daily to intermittent weekly schedules. For pediatric dosing, some practitioners use half of the adult dose for children of average weight and one-quarter of the adult dose for infant patients; however, it is not clear if this is necessary. Many products contain package labels that state “through end of shelf life,” which suggests the minimum CFU remains in the product if it is consumed before the end of the shelf life versus “at the time of manufacture,” which indicates the maximum CFU that a consumer can expect to obtain from the product.

### Quality control of products

Different studies have noted variability in the quality of over-the-counter probiotic products. For example, a cross-sectional analysis of 14 commercial probiotic products revealed that many of the products in the sample contained unadvertised additional lactobacilli and bifidobacteria cultures, whereas other products were devoid of species that were listed on the product label. Thus, the label claims on some probiotic products may or may not represent the true constituents of over-the-counter products.<sup>89</sup>

### Effect on patient adherence

Data from clinical trials suggest that rates of patient adherence to medication are estimated to range between 43% and 78%.<sup>90</sup> As the complexity of the medical regimen increases, the likelihood of adherence decreases. Adding a probiotic supplement can potentially positively affect adherence (e.g., adding a probiotic supplement to prevent antibiotic-associated diarrhea)<sup>91</sup> and patient outcomes. This is due to the ability of probiotics to ameliorate some antibiotic-associated discomfort/side effects, as well as their potential to improve antibiotic efficacy. As a result, counseling and education are essential components of probiotic therapy.

### Safety considerations

Specific probiotic strains are generally regarded as safe and are available over the counter. Historically, lactobacilli and bifidobacteria associated with food have been considered as safe.<sup>92</sup> Like other probiotics, they are normal commensals of the GI tract and their safety has been demonstrated in a variety of foods and dietary supplements. Because probiotics are viable microorganisms, they have the potential to cause invasive infections in hosts who may have compromised mucosal epithelia. Large-scale use of *L. rhamnosus* in Finland has not been shown to result in an increased infection rate<sup>93,94</sup>; however, cases of probiotic-related infection, including bacterial sepsis and fungal sepsis, have been reported.<sup>95</sup>

Probiotics may theoretically be responsible for four types of side effects: systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, and gene transfer. Therefore, probiotics should be used with caution in children, elderly persons, and individuals with major risk factors or multiple minor risk factors. Furthermore, since probiotics may be derived from many different genera and species beyond *Lactobacillus* and *Bifidobacterium*, it is important that safety not be presumed and that the specific nature of any probiotic be considered during a safety evaluation.

## CONCLUSION

Understanding of the gut microbiota's role in nutrition, health, and disease has increased significantly since 2000. Recently developed technology has been used to explore the transcriptional profiles and genome differences of a variety of microorganisms, allowing a better understanding of how they are metabolized after consumption, as well as their composition and activity in the GI tract. The appropriate selection of probiotic strains forms the basis for further development of supplements and food products, as well as for planning future clinical trials. In vitro studies are useful for evaluating the safety and efficiency of probiotic strains; however, they are not sufficient for recommending the use of probiotic strains in vivo. Recent advances in science have revealed many mechanisms by which probiotics exert health-promoting effects in humans and laboratory animals. Probiotics and prebiotics have been reported to aid in the treatment of many dysfunctions of the GI tract, including immunoinflammatory disorders, and in the prevention of some infectious diseases. There are promising hypotheses that suggest probiotics and prebiotics may aid in the prevention of obesity and type-2 diabetes. However, further research is needed to elucidate the functional aspects of probiotics in foods and dietary supplements and how they impact human health in relation to various disorders and/or overall well-being.

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The International Scientific Association for Probiotics and Prebiotics (ISAPP) is an association of academic and industrial scientists from all pertinent disciplines involved in research on fundamental and applied aspects

of probiotics and prebiotics. Its activities focus on science, not the promotion of specific commercial products (<http://www.isapp.net>).

The American Gastroenterological Association (AGA) is the trusted voice of the GI community, with 17,000 members worldwide involved in all aspects of the science, practice, and advancement of gastroenterology. The practice, research, and educational programs of the organization are administered by the AGA Institute (<http://www.gastro.org>).

*Declaration of interest.* Mary Ellen Sanders consults with numerous food and dietary supplement companies in the area of probiotic microbiology, but she does not have any stock, stock options, partnership shares, or other ownership interest in any of these entities. The other authors have no relevant interests to declare.

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