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New Scientific Paradigms for Probiotics and Prebiotics

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Abstract

The inaugural meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP) was held May 3 to May 5 2002 in London, Ontario, Canada. A group of 63 academic and industrial scientists from around the world convened to discuss current issues in the science of probiotics and prebiotics. ISAPP is a non-profit organization comprised of international scientists whose intent is to strongly support and improve the levels of scientific integrity and due diligence associated with the study, use, and application of probiotics and prebiotics. In addition, ISAPP values its role in facilitating communication with the public and healthcare providers and among scientists in related fields on all topics pertinent to probiotics and prebiotics. It is anticipated that such efforts will lead to development of approaches and products that are optimally designed for the improvement of human and animal health and well being. This article is a summary of the discussions, conclusions, and recommendations made by 8 working groups convened during the first ISAPP workshop focusing on the topics of: definitions, intestinal flora, extra-intestinal sites, immune function, intestinal disease, cancer, genetics and genomics, and second generation prebiotics.

Key Words: probiotics, prebiotics, guidelines, intestine, urogenital immunity, genetics

Humans have evolved in symbiosis with an estimated 10^{14} resident microorganisms. However, as medicine has widely defined and explored the perpetrators of disease, including those of microbial origin, it has paid relatively little attention to the microbial cells that constitute the most abundant life forms associated with our body. Microbial metabolism in humans and animals constitutes an intense biochemical activity in the body, with profound repercussions for health and disease. As understanding of the human genome constantly expands, an important opportunity will arise to better determine the relationship between microbial populations within the body and host factors (including gender, genetic background, and nutrition) and the concomitant implications for health and improved quality of life. Combined human and microbial genetic studies will determine how such interactions can affect human health and longevity, which communication systems are used, and how they can be influenced to benefit the host.

Probiotics are defined as "live microorganisms which, when administered in adequate amounts confer a health benefit on the host."[1] The probiotic concept dates back over 100 years, but only in recent times have the scientific knowledge and tools become available to properly evaluate their effects on normal health and well being, and their potential in preventing and treating disease. A similar situation exists for prebiotics, defined by this group as "nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria." Prebiotics function complementary to, and possibly synergistically with, probiotics. Numerous studies are providing insights into the growth and metabolic influence of these microbial nutrients on health. Today, the science behind the function of probiotics and prebiotics still requires more stringent deciphering both scientifically and mechanistically. The explosion of publications and interest in probiotics and prebiotics has resulted in a body of collective research that points toward great promise. However, this research is spread among such a diversity of organisms, delivery vehicles (foods, pills, and supplements), and potential health targets such that general conclusions cannot easily be made. Nevertheless, this situation is rapidly changing on a number of important fronts.

With progress over the past decade on the genetics of lactic acid bacteria and the recent,[2,3] and pending,[4] release of complete genome sequences for major probiotic species,

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the field is now armed with detailed information and sophisticated microbiological and bioinformatic tools. Similarly, advances in biotechnology could yield new probiotics and prebiotics designed for enhanced or expanded functionality. The incorporation of genetic tools within a multidisciplinary scientific platform is expected to reveal the contributions of commensals, probiotics, and prebiotics to general health and well being and explicitly identify the mechanisms and corresponding host responses that provide the basis for their positive roles and associated claims.

In terms of human suffering, the need for effective new approaches to prevent and treat disease is paramount. The need exists not only to alleviate the significant mortality and morbidity caused by intestinal diseases worldwide (especially diarrheal diseases in children), but also for infections at non-intestinal sites. This is especially worthy of pursuit in developing nations where mortality is too often the outcome of food and water borne infection. Inasmuch as probiotics and prebiotics are able to influence the populations or activities of commensal microflora, there is evidence that they can also play a role in mitigating some diseases.[5,6] Preliminary support that probiotics and prebiotics may be useful as intervention in conditions including inflammatory bowel disease, irritable bowel syndrome, allergy, cancer (especially colorectal cancer of which 75% are associated with diet), vaginal and urinary tract infections in women, kidney stone disease, mineral absorption, and infections caused by *Helicobacter pylori* is emerging. Some metabolites of microbes in the gut may also impact systemic conditions ranging from coronary heart disease to cognitive function, suggesting the possibility that exogenously applied microbes in the form of probiotics, or alteration of gut microecology with prebiotics, may be useful interventions even in these apparently disparate conditions. Beyond these direct intervention targets, probiotic cultures can also serve in expanded roles as live vehicles to deliver biologic agents (vaccines, enzymes, and proteins) to targeted locations within the body.

The economic impact of these disease conditions in terms of diagnosis, treatment, doctor and hospital visits, and time off work exceeds several hundred billion dollars. The quality of life impact is also of major concern. Probiotics and prebiotics offer plausible opportunities to reduce the morbidity associated with these conditions.

The following addresses issues that emerged from 8 workshops (Definitions, Intestinal Flora, Extra-Intestinal Sites, Immune Function, Intestinal Disease, Cancer, Genomics, and Second Generation Prebiotics), reflecting the current scientific state of probiotics and prebiotics. This is not a comprehensive review, however the study emphasizes pivotal knowledge gaps, and recommendations are made as to the underlying scientific and multidisciplinary studies that will be required to advance our understanding of the roles and impact of prebiotics, probiotics, and the commensal microflora upon health and disease management.

DEFINITIONS AND USE

The term "probiotic" remains undefined legally in many countries, and regulatory approaches differ among countries worldwide. Diverse categories encompass probiotic products, including: food, functional food, novel food, natural remedy (Denmark Sweden and Finland), natural health product (Canada), dietetic food (Italy), dietary supplement (USA), biotherapeutic and pharmaceuticals (probiotic pharmaceuticals are available in Canada, China, eastern European countries, France, Germany, Belgium, Austria and Italy). There is no official definition of probiotic in Japanese regulation, but several probiotic and prebiotic products have achieved FOSHU (foods for specialized health use) status, with health statements being approved by the Japanese Ministry of Health.

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have recently collaborated to establish guidelines for the use of the term "probiotic" in foods and levels of evidence necessary to make a health claim.[6] Their recommendations will be considered by Codex in terms of labeling and claims for foods. In an effort to harmonize the term, the definition of probiotics stated earlier appears to reflect current scientific as well as commercial understanding and usage of probiotics. The term "health" in the definition may be appropriately replaced by "physiological" as the phrase "beneficial physiological effect" is inclusive of both health and functional effects. Functional effects would include endpoints such as quality of life indices, fecal microbial alterations, cholesterol lowering, immune modulation, and metabolic markers. Furthermore, this definition requires that the term "probiotic" only be applied to live microbes having a substantiated beneficial effect. Thus, microbes administered alive are considered probiotics regardless of their ability to survive intestinal transit. As such, strains or species that do not survive intestinal transit, such as *S. thermophilus*, for example, could be considered probiotic. Although a preparation of non-viable bacteria may mediate a physiologic benefit, they are not considered to be "probiotics" under the present definition, and terms such as nonabiotic or abiotic may be considered for such preparations.

Another implication of the FAO/WHO definition is that unless strains are shown to confer clinically established physiological benefits, they should not be referred to as probiotics. In vitro tests, while useful to gain basic knowledge of probiotics and prebiotics, to establish mechanisms of action, or justify expanded clinical evaluation, cannot be assumed to predict functionality in the human body, and are thus insufficient substantiation for use of the terms "probiotic" or "prebiotic". Appropriate and substantiated use of

these terms will elevate credibility of probiotics among scientists, physicians, and consumers.

The principal organisms in use as probiotics are *Lactobacillus* and *Bifidobacterium*. These genera initiated their role as probiotics through their association with healthy human intestinal tracts and, in the case of lactobacilli, their presence in the human diet through fermented foods. However, other genera including *Escherichia*, *Enterococcus, Bacillus,* and *Saccharomyces* are also used, based on documented efficacy through clinical studies. The basis for a microbe being termed a "probiotic" should be proven efficacy and safety under the recommended conditions of use,[1,6] with consideration given to target population, route of administration, and dose applied.

Nomenclature of probiotic bacteria must conform to current, scientifically recognized names (http://www.bacterio.cict.fr/). Protracted use of older or misleading nomenclature, such as *Lactobacillus sporogenes*, is not acceptable on product labels. DNA–DNA hybridization is the reference method to specify that a strain belongs to a species, but sequencing of DNA regions encoding species-specific areas of the 16S rRNA is a preferred substitute in combination with phenotypic confirmatory tests such as fermentation of a range of sugars. Strain typing has to be performed with a reproducible genetic method and all strains should be deposited in an internationally recognized culture collection. In the near future, it is completely likely that genome sequencing may be justifiable as a requisite for new strain introduction.

The definition of a prebiotic, presented above, may overlap with dietary fiber (a whole food) or "added fiber", although most fibers including pectins and xylans are not selectively fermented. Deliberately, this definition widens the application of the original definition, which related prebiotics to colonic bacteria only. Hence, prebiotics can now be considered to act in other areas of the gastrointestinal tract such as the mouth, stomach, and small intestine, as well as non-intestinal sites, such as the vagina and skin.

Carbohydrates said to be prebiotics have been variably tested for modulating activities of the gut flora. For example, fructooligosaccharides, galactooligosaccharides and lactulose are recognized for their bifidogenic effects in laboratory, animal, and human trials carried out in multiple centers. These substances appear to be the most used in the current market. In Japan, a much wider list of prebiotics exists, which includes soyoligosaccharides, xylooligosaccharides, isomaltooligosaccharides, gentiooligosaccharides, lactosucrose and glucooligosaccharides. These are currently being tested in Europe and elsewhere for health-promoting attributes. Resistant starches and some sugar alcohols have also been proposed as prebiotics. New prebiotics, for example those derived through enzymatic procedures, from dietary fibers such as oligosaccharides of pectin, xylan and cellulose, and as anti-adhesive forms (blocking pathogen binding and providing nutrients for lactobacilli and bifidobacteria) with multiple functionality are under development. With new advances in molecular based diagnostic procedures for characterizing the response of the gut flora to dietary change, a more reliable database of effects should ensue.

To optimize the use of prebiotics, studies are required to determine their impact upon relative growth of different genera, species and strains of putatively desirable bacteria. To help define how prebiotics function, there is a need for structure to function studies. A selective fermentation is one requirement for an efficient prebiotic, with certain oligosaccharides seemingly specifically stimulating the bifidobacteria. However, it is not clear why this is the case or why certain linkages induce selective changes in a mixed microbial ecosystem. As additional information on the biochemical, physiological and ecological capabilities of target organisms is generated, such relationships will become more apparent. Such studies should use multi-species biofilm models, such as multiple stage chemostats and in vitro tests, or animal models designed for the analysis of the immune modulation capacity of probiotic strains. Functional biomarkers (organic acids, various enzymes) need to be monitored and mRNA microarrays or proteomic chip systems used to identify key changes following probiotic and prebiotic administration. Rapidly evolving metabolomic approaches should also uncover relevant biomarkers.

A synbiotic is defined as "a product that contains both probiotics and prebiotics." [7] That is, the prebiotic may function to fortify survival, growth, or metabolism of the probiotic in vivo, if the correct probiotic/prebiotic combination is used. However, one implication of this definition is that demonstration of a synergistic effect of the probiotic and prebiotic comprising the synbiotic is not a requirement. Individual components of the synbiotic may themselves exert independent health benefits.

The FAO/WHO has recommended that physiological benefits of probiotic foods be substantiated with a phase 2, double blind, randomized, placebo-controlled trial or appropriate equivalent.[8,9] Human studies are essential before a physiological benefit for humans is projected or claimed for either probiotics or prebiotics. The strength of the claim should be correlated to the level of the evidence. The difficulty of identifying health benefits in healthy persons suggests the value of functional markers for substantiating health effects. Standards for Good Clinical Practice have been delineated by the International Committee on Harmonization (www.ifpma.org/ich1.html) for pharmaceutical agents, but they could also be applied to human research on probiotics and prebiotics.

LABELING

While the marketing of so-called "probiotic" products has occurred for some time, there is little or no enforced

worldwide regulation regarding labeling for quality or efficacy. In the United States, a few probiotic products have been removed from the market because labeling reflected non-allowable disease claims. A disease claim in this context refers to statements relating to the use of probiotics to diagnose, prevent, treat, cure, or mitigate a disease. Some products mislabeled based on inaccurate use of nomenclature for genus and species, inaccurate cell count and/or with unsubstantiated structure/function statements continue to be sold worldwide.[9–12] A structure/function statement in this context refers to statements relating to the use of probiotics to improve the normal functioning of the human body. A similar situation also applies to prebiotics where the active ingredient and its concentration are often not clearly or accurately stated on the label. Accumulation of scientific evidence that defines mechanisms of action, elevates manufacturing standards, and accurately labels probiotic and prebiotic products is essential to building a solid platform that will support continued growth and development in this field.

From a scientific perspective, the suitable description of a probiotic product as reflected on the label should include:

- Genus and species identification, with nomenclature consistent with current scientifically recognized names
- Strain designation (eg, GG)
- Viable count of each strain at end of shelf life
- Recommended storage conditions
- Safety under the conditions of recommended use
- Recommended dose, which should be based on induction of the physiological effect
- An accurate description of the physiological effect, as far as is allowable by law
- Contact information for post-market surveillance

As indicated above, all probiotics should be identified with a strain designation, and phenotypic and genotypic patterns should both be determined. With the current availability of high throughput sequencing, it is encouraged that the genomes of all commercial probiotic cultures be sequenced and their genetic content established for safety and functionality. This is also important for strain documentation, tracking, product consistency and quality control, post-market surveillance, and all research efforts, including human studies. Furthermore, as the current state of evidence supports the strain dependency of functional effects, strain identification is essential. In addition, all commercial probiotic strains should be deposited in a collection recognized by the International Depository Authority.

Labeling of prebiotics should also include information on source and specific dose of active component based on levels documenting the selective impact on certain indigenous bacteria and physiological benefits on the host.

GENETIC ANALYSIS AND ENGINEERING

Analytical tools have become available recently to investigate in depth the genetics of probiotic organisms and related bacteria important to the fermentation industry. Realizing their practical significance in fermentation, bioprocessing, agriculture, food, and more recently, medicine, the lactic acid bacteria (LAB) have been the focus of intense genomic research. The first complete genome of the LAB group was published on *Lactococcus lactis* subsp. *lactis* IL1403.[13] Its publication was a significant milestone for LAB researchers and the data yielded some unexpected findings. Analysis of the 2.4 Mb genome revealed: biosynthetic pathways for all 20 amino acids, although not all were functional; a complete set of late competence genes; 5 complete prophages; partial components for aerobic metabolism; and a wealth of ATP-binding cassette transporters reflecting the organism's fastidious lifestyle. Noting that some of these systems are not functional or complete, the genomic analysis of *Lactococcus* spp. suggests an evolutionary trend toward minimization of the chromosome and elimination of unnecessary systems during adaptation to nutritionally complex environments. Similar exploration into the genomes of lactobacilli and bifidobacteria will likely contribute to our understanding of the ecology, phylogeny, metabolic capacity, and pathogenic potential of probiotics.

The genomes of 2 probiotic species have been completed *Bifidobacterium longum*[3] and *L. plantarum*[2] with 8 more nearing completion: *L. johnsonii*, *L. acidophilus*, *L. gasseri, L. casei* (2 strains), *L. rhamnosus*, *B. longum* (a second strain) and *Bifidobacterium breve*. Genome information is rapidly becoming available in the public domain through publication and the appearance of draft genome sequences in 2002 provided by the United States Department of Energy-Joint Genome Institute (JGI) in collaboration with the Lactic Acid Bacterial Genomics Consortium. An exciting set of discoveries are already apparent, for example revealing fimbriae like structures on *B. longum*, and the presence of gene regions in *L. plantarum* that promote a flexible lifestyle in habitats ranging from green plants to the human GI tract.

As part of their microbial genomes program (see http://www.jgi.doe.gov/JGI_microbial/html/index.html) 11 additional genomes are being sequenced in collaboration with the Lactic Acid Bacteria Genome Consortium (LABGC). Of the genomes being sequenced, 3 represent probiotic species (*L. gasseri, L. casei,* and *B. longum*), and 3 others (*L. lactis, S. thermophilus,* and *L. delbrueckii*) represent organisms that may be potentially used as intestinal delivery vehicles for certain biologics. As these sequences are generated, they will be available on the JGI website for public use. Timely, public availability of genome information for various LAB species will catapult the field's collective efforts to carry on with comparative and functional

genomic analyses of probiotic species within the LAB group.

Genomic regions, identified through genome sequencing, that may help identify regions critical to the survival and functionality of commensal or probiotic organisms in their corresponding habitats might include:

- Conserved versus distinct gene sets
- Genes resulting from recent horizontal transfer
- Altered GC content–islands/regions of adaptability (surrounding prophages, IS elements; exopolysaccharides, bacteriocins, transposons)

Gene-based studies will contribute significantly toward our understanding of probiotic bacteria and the influence of prebiotics on the commensal flora. Some opportunities for study include comparison of the genetic content and organization of probiotic organisms against the growing number of genomes from commensal and pathogenic organisms (eg, *Bacteriodes spp., Streptococcus mutans, Streptococcus bovis, Streptococcus pneumoniae, Clostridium spp.,* and *Listeria monocytogenes*).[4] This analysis is expected to reveal key similarities and differences that reflect both the habitat occupied and the lifestyle within these habitats.

Our viewpoint will be augmented considerably by comparisons of closely related species, occupying similar versus dissimilar habitats, multiple genomes of the different strains within the same species, and multiple genomes of strains found in various environments. Other studies could explore how probiotics and commensal organisms selectively catabolize prebiotics and potentially reveal metabolic pathways induced during their growth. Prebiotic use could be focused through genomics via definition of functional targets.

In 2003, a greater collection of genome sequences will be publicly available, but even then the sequences are not likely to reflect the biodiversity that occupies these complex ecosystems. As a result, expanded sequencing capacity will continue to support genomic efforts to determine the microbiomes of microorganisms inhabiting the mouth, vagina, and distinct regions within the intestinal tract.[4,14,15] Viewing the metagenome, which is defined as the "collective genomic content of a diverse 'cell-wall less' population within an environment," is likely to reveal key functions essential for survival, competition, and activity of commensals in that environment. This wealth of information will serve as the matrix upon which science can examine the interactions, roles, and impact of probiotic cultures on the microflora. This area promises to be one of the most exciting frontiers of science in the decade ahead. As these data accumulate, one major challenge will be continuous updates of genomes and genome sequences as each new microorganism is sequenced. The quality of the bioinformatic view, essential to deciphering probiotic mechanisms and functional roles, will rely heavily upon continuously up-dated databases and comparative analyses.

Over the past decade, efforts in plasmid biology and biotechnology of LAB have supported the development of genetic tools (eg, transformation systems, cloning and expression vectors, integration vectors, and systems for gene inactivation) in a select number of probiotic cultures, that are relatively well developed commercially or scientifically.[16] There remains, however, many model probiotic strains that are so far recalcitrant to genetic engineering. Genetic accessibility is an important selection trait to consider for any new probiotic strains, recognizing the powerful impact that genomic information and resulting approaches will play in establishing gene function and the mechanistic basis of functionality. For example, recombinant strategies such as the in vivo expression technology (IVET) and signature tagged mutagenesis (STAG),[17] are designed to identify and investigate gene regulation and function in vivo. These techniques have been used extensively to study host–pathogen-host interactions, and they have more recently been used to study probiotics[18] and other beneficial organisms in various habitats.[19]

Genomic regions predicted to be important for colonization, survival, functionality and safety include loci encoding the following traits:

- Acid tolerance
- Bile tolerance
- Stress tolerance
- Surface proteins
- Lipoteichoic acid biosynthesis
- Extracellular proteins
- Exopolysaccharide biosynthesis
- Adherence factors
- Putative virulence factors
- Aggregation
- Biofilm formation
- Immunomodulation
- Bacteriocin production
- Carbohydrate (prebiotic) utilization and metabolism
- Gene transfer potential
- Antibiotic resistance
- Putative virulence factor homologs
- Siderophores, scavengers of Fe^{++}
- Quorum sensors and response regulators
- Prophages, prophage remnants, lysogenic conversion characters
- Mobile genetic elements

Functional genomic analyses of these properties will create opportunities to establish direct cause and effect relationships, but it is also expected that global, pleiotrophic, and cascading effects will result from some gene knockouts. Redundant proteins encoded in the genome are also expected to have cumulative effects that are not resolved by

a 1-gene, 1 phenotype analysis (ie, there may be hundreds of surface proteins that impact immunomodulation, attachment, agglutination, and retention).

SAFETY

Probiotic lactobacilli and bifidobacteria have been used in food products and dietary supplements for decades, with a compelling record for safe consumption.[20,21] To assure safety, considerations should include potential contraindications for the target consumer, proven history of safe use for the recommended dose and route of administration, frequency of association of the species (or strain) with infection, likelihood of production of deleterious metabolic endproducts, association with transferable antibiotic resistance, sensitivity to therapeutic antibiotics, and relatedness to species that produce hemolysins or mammalian toxins.[6,20] Consideration should be given to the condition of the consumer or patient, since those with underlying disease, at high risk for translocation (eg, undergoing radiation therapy or with bloody diarrhea), and immunosuppressed or recovering from oral or GI surgery (especially short bowel loop), could have increased susceptibility to infections such as endocarditis, septicemia, or liver abscess. These latter considerations also apply to prebiotic-based interventions.

Tolerance tests in animals for species of *L. rhamnosus*, *L. helveticus, L. bulgaricus,* and *B. longum* have shown that these bacteria are tolerated at levels greater than 6 g/kg body weight.[22] *L. rhamnosus*, *L. acidophilus* and *B. lactis* have also been tested in tolerance studies and shown to be safe at 50 g/kg/d for a mouse, which extrapolates to 35 g/d for a 70 kg person.[23] Consensus among scientists on the most useful bioassays to assure safety of probiotic strains would be an important future recommendation, as this has not hitherto been assembled.

Because many probiotics can be used in foods as well as pharmaceuticals or supplements, there have been efforts to grapple with issues surrounding safety of microbes that have a history of safe use in foods. Recently, the International Dairy Federation (http://www.fil-idf.org/) in collaboration with the European Food and Feed Cultures Association assembled a list of microorganisms with a documented history of safe use in food. This list can be viewed at http://www.effca.org/anglais/pages/id_title_15.htm. The inventory is not considered exhaustive, but provides a starting point for establishing a rationale for safety of microbes used as probiotics. In the USA, the FDA publishes a "Partial List of Microorganisms and Microbial-Derived Ingredients that are used in Foods", which includes approved food additives, substances whose GRAS status has been affirmed by FDA, and substances that the FDA listed as GRAS based on a history of safe use in food (prior to 1958). This list is also not considered to be complete and has limited utility in that many of the microbes used currently as probiotics are not included (only *L. bulgaricus, S. thermophilus* and *L. acidophilus* are listed).

Considering the safety of prebiotics, one key side effect is excessive gas production, perhaps leading to distension difficulties. The usual target organisms for prebiotic intake (bifidobacteria and lactobacilli) do not produce gas as part of their normal metabolism. Hence, if such difficulties occur then the prebiotic dose is probably too high and selectivity of the fermentation is being compromised. Other safety aspects that should be taken into account for prebiotics include effects on gut transit time, osmotic regularity, and satiety.

DOSAGE

While few studies have established the minimum effective dose of a probiotic to convey a physiological effect, probiotic-induced changes are rarely seen at daily doses of less than 10^{8-10} colony forming units (cfu).[24] However, one can only speculate as to how many probiotic cells reach target sites alive. Probiotic bacteria that are tolerant to acid stress would be expected to survive well during stomach passage. Delivery of the probiotic in an encapsulated form, or in a stabilizing food matrix could also enhance survival (25). Therefore, a meaningful discussion of required dose for an effect would necessitate clear definition of innate and in vivo factors that influence probiotic stability. Delivery systems that could stabilize probiotic cells during encounter with detrimental conditions in the mouth, nasopharynx, stomach, intestine, vagina and other sites, may reduce the dose of viable cells needed for an effect. It may also allow better use of prebiotics, which currently tend to be effective only in the lower part of the alimentary tract. Effective doses of prebiotics seem to be about 1 to 3 g/d for infants and 5 to 15 g/d in adults. As prebiotics are generally mixtures of different chain length carbohydrates, the dose of the active components can be difficult to determine and additional studies are needed.

MOLECULAR AND CELLULAR IMMUNE RESPONSE

Gnotobiotic animal studies have shown that the commensal flora has immunostimulatory properties.[26] What is not clear however, is the extent to which antigenic components of bacterial cell walls modulate the immune system to establish a stable association between an animal host and its resident microbiota. There are few reports of systematic investigation of host cell responses to distinct commensal-associated molecular patterns (CAMPs) of probiotic strains. Similarly, even when probiotics have been administered immediately after birth,[27,28] the long term fate of the organisms and host responses to their later ingestion have not been reported. This is unfortunate, as such studies would provide insight into several important factors, namely

whether in this situation the strains can persist for extended periods in the host, whether immunotolerance occurs, and if heightened states can be exerted to elicit anti-inflammatory, anti-pathogen, and anti–carcinogenic responses.

It is commonly suggested that probiotics must "persist and multiply" to be effective. However, while conclusions have been based on fecal and not mucosal biopsy material, a number of studies have now shown that ingested probiotic strains do not necessarily become established members of an already formed microflora (for consistency). Rather, they persist only during periods of dosing and for a short time after feeding is halted.[29,30] This behavior has been anticipated as probiotics are considered allochthonous microflora, existing temporarily, versus the permanent autochthonous flora found to be present at relatively high levels throughout life.[5,30] The allochthonous nature of probiotics may reflect many factors, including possibly the absence of permanent host-bacterial receptors or the organisms' inability to compete, permanently, with the residing autochthonous flora that may exist in close (biofilm-like) association with the epithelial layers of the intestine. The initial stages of immune development, namely in neonates, shows that responses to environmental antigens are generally skewed toward a T_H2-type cytokine profile, which typifies allergic diseases.[31,32]

Allergic diseases have increased substantially in developed countries during recent decades, a situation which has led to the formulation and promotion of the "hygiene hypothesis".[33] This ascribes the increase in allergic disease to an increased emphasis on hygiene, which reduces the exposure of neonates to microbial stimuli thereby favoring immune responses toward a T_H2 versus a T_H1 cytokine profile.[34,35] Intestinal colonization with commensal bacteria is critical for the establishment of oral tolerance.[36] This in turn has heightened interest in the potential use of probiotics in neonates to prevent allergic diseases from developing in later life. Supporting this focus is a recent clinical study, which demonstrated a highly significant reduction in the frequency of atopic eczema in 2-year-old children who as newborns were nursed by their mothers and received a *Lactobacillus* supplement.[28] The jury is still out on the hygiene hypothesis, with for example one study of a patient pool of 20,050 with allergic and autoimmune disease history showing "no evidence of an inverse relationship between atopy and patient reports of physician-diagnosed common autoimmune disorders," [37] whereas another study of the cytokine patterns, produced from cord blood mononuclear cells relative to adult cells after stimulation with bacterial strains from the normal flora, supported the hypothesis.[38]

The wide range of existing animal models, particularly transgenic knockout mice with specific cellular or molecular deficiencies (eg, B- and T-cell deficient animals), have not been used extensively to investigate immunologic responses to either commensal or probiotic bacteria. Neither have they been applied to monitoring immune responses to prebiotic metabolism. A number of key questions or goals may be addressed currently with these models including: (1) identification of the developmental windows most sensitive to immunologic manipulation; (2) the selection of well defined immunogenic versus tolerogenic probiotic strains; and (3) the identification of standard immunologic biomarkers that could be measured in human clinical studies, including the effects of prebiotics.

By administering probiotic strains early in life, they may have the opportunity to interact with host cell receptors early, establish apically on epithelial and mucosal surfaces and potentially establish an autochthonous condition. Such selective colonization will depend upon availability of receptor sites, competition for space and nutrients, and interactions with other microbes entering the gut. This approach raises several issues. As with the case of successful repression of atopic dermatitis, it might be possible to "program" the host to be at lower risk of disease. Colonizing our bodies from birth relies on chance and environmental circumstance provided largely by the mother. No effort has been made to direct or program the initial microbe exposure. The short and long term effects of such efforts on the autochthonous microflora would be revealing. Large long-term studies in different continents comparing genetic background, gender, diet, and other factors are also needed to determine the extent to which the microflora influences longevity and quality of life. The potential significance could be enormous and can likely only be addressed by clinical studies coordinated through large national or international funding programs.

For prebiotic and probiotic-induced immune modulation to take place once the intestinal flora and immune system is developed, immune cells are endowed presumably with recognition receptors or are otherwise sensitive to probiotic-specific structures and catabolites. There is no *a priori* reason that introduced strains would need to persist and multiply to encounter intestinal immune cells. Given the diversity of inflammatory or immune responses that can be mounted by the intestinal epithelium, a simple association of probiotics with the epithelium might be sufficient to trigger signaling cascades that would ultimately activate underlying immune cells in the lamina propria. A large number of in vitro studies have demonstrated the ability of probiotic strains to up-regulate a variety of cytokines and bioactive molecules. However, the work rarely relates back to a particular physiologic or pathologic condition, the real conditions encountered by organisms at that site, or the bioactive features of the probiotic cells responsible for signaling.

Modulation of host "immunity" is one of the most commonly purported benefits of the consumption of probiotics. However, general claims vastly over-state current knowledge of both the fate of ingested probiotic products and their specific effects on molecular and cellular components of the

immune system. Furthermore, daily ingestion of the same probiotic strain(s) is unlikely to retain a "boosting" effect. Therefore, the duration of probiotic exposure needs to be better understood in terms of immunity and tolerance. The following represents some gaps in this knowledge and some potential ways to fill them.

The means by which organisms entering the gut (or other colonized mucosal surfaces such as the vagina and oral cavity) interact with the mucus barrier, translocate and influence pro- and anti-inflammatory cytokines, NK cells, dendritic cells, macrophage, and antibody production remains to be determined. Would the answer be forthcoming if it were possible to follow ingested bacterial cells (perhaps by fluorescent labeling of the organisms), measure genomic and protein expression changes within epithelial and immune cells at the bacterial interface, and determine the effect on peripheral immunity? Human studies in more "controlled" environments such as an ileal conduit, vagina, or rectal pouch could be insightful, but animal knock-out models are still required to control microflora content, diet, host genetics, and immunity.

One key consideration is the effect of bacterial adhesion on translocation across the epithelium. Routine translocation of commensal bacteria to mesenteric lymph nodes has been clearly demonstrated,[39–42] and presumably is central to the developmental activation of the intestinal immune system. Furthermore, among a variety of intestinal bacteria, an inverse relationship has been demonstrated between the degree of adhesiveness and degree of translocation (RD Berg, personal communication). It appears that "physiologic translocation" (ie, to MLNs) is a more desirable trait of candidate probiotic strains than adhesion to epithelial surfaces. On the other hand, bacterial adhesion to M cells covering Peyer patches would be expected to enable the activation of IgA responses, which depending on context might be a desired outcome. This area of research is in critical need of further investigation. Unfortunately, M cells cannot be propagated in primary culture and physiologically relevant M cell lines do not exist. Recombinant strains genetically designed to target M cells and which are labeled by one means or another for in situ identification will be required to initiate investigation of M cell and host IgA responses to probiotic organisms. These studies should also employ transgenic mouse strains in which specific immune components have been genetically ablated to systematically define the cellular and molecular basis of host responsiveness to probiotics.

Genetically-tagged bacterial strains will be crucial for determining the regions of the gastrointestinal tract that are most immunologically responsive to ingested probiotic strains or prebiotic based stimulation, another key consideration that is fully undefined at present. Given the central role of Peyer patches for the development of secretory IgA, it follows that probiotic strains targeting M cells should be identified for applications that seek to bolster intestinal immunity. On the other hand, probiotic strains with an affinity for the colonic epithelium and also possessing anti-inflammatory properties are likely key for the treatment of large intestinal inflammatory disorders, such as ulcerative colitis. At this stage however, these are only theoretical considerations, as the availability of standardized reagents and experimental conditions are generally not in place for empirical research.

Synbiotics also contain live microorganisms and therefore the immunologic considerations described earlier for probiotics are also relevant for these products. Prebiotic fructooligosaccharides, galactooligosaccharides, and lactulose appear to selectively stimulate certain bifidobacteria,[43] but the consequences of short or long term gut microflora modulation are still unclear, including immune effects.

EFFECT ON GASTROINTESTINAL HEALTH

Gastroenterologists once defined gastrointestinal health as the absence of chronic disorder such as inflammatory bowel disease (IBD). However, this is too restrictive. The increasing frequency of digestive functional disorders, including non-ulcer dyspepsia and irritable bowel syndrome (IBS), justifies an enlargement of a definition that includes intestinal well-being and the overall impact on quality of life including reduction of disease risk. Some animal studies are required to investigate this concept.

The intestinal microflora has been linked with a number of intestinal diseases including colon cancer, (IBS) and IBD,[44–47] however few details of their involvement have been elucidated. Since colon cancer and IBD can lead to extreme therapeutic approaches, including surgical excision, clarification of the role of the microflora in these diseases may significantly reduce morbidity.

There are 2 broad categories of experimental animal models of IBD—one in which the disease is induced by exposure to chemical (eg, indomethacin, acetic acid, TNBS, DSS) or microbial (eg, chemotactic peptides) agents, or the other by lymphocyte transfer (eg, CD45RB T cells in SCID mice), where the disease arises naturally after genetic selection or transformation (eg, HLA-B27 transgenic rats, IL10 knockout mice). Several studies have shown interesting effects of probiotics and prebiotics on IBD and permitted further insight into the mechanisms of action. In particular, the use of probiotics in the IL-$10^{-/-}$ mice resulted in a complete normalization of physiological transport function and barrier integrity in conjunction with a reduction in mucosal secretion of TNF-alpha and IFN-gamma.[48] Interestingly a multi-strain probiotic appears to have promise for clinical effect in Crohn disease[49] while a single *Lactobacillus* strain did not.[50] Notably, recombinant *L. lactis* strains secreting IL-10 were recently demonstrated to have a preventive or therapeutic effect in 2 different mouse models of

colitis.[51] Information on the potential role of prebiotics in alleviating IBD is currently sparse, but it is emerging.

The health of the intestinal tract can be monitored in terms of stool production, transit time, pain, discomfort, sensitivity to distension, gas transit, and blood in the stool. Additionally, but more difficult to assess, properties such as permeability (as measured by urinary excretion of orally administered substances or imaging for leakage of labeled compounds) and bacterial translocation (measured by finding intestinal organisms at distant sites) are considered important indices of gut function. Although the presence of pathogens is often associated with disease, the microflora of a healthy intestinal tract is difficult to define. Defensins, antimicrobial peptides localized in epithelia and released at mucosal surfaces,[52] may influence the microflora composition, but their level in different patient sub-groups is unknown. The ability of probiotics and prebiotics to influence these factors requires further study. With respect to influencing mucus production by intestinal cells, in vitro experiments indicate that this can be achieved through signaling processes from lactobacilli to the cells.[53]

GENETICS AND GENOMICS

In recent years, advances in molecular technologies based on rRNA have illuminated the diversity of the gut microbiota. rRNA gene sequencing studies have revealed the presence of species previously unrecognized as components of the human intestinal tract. The ultimate aim is to characterize the microflora "at a glance." Technologies available, include genetic probing strategies by microscopy, image analysis or flow cytometry, microarray, genetic fingerprinting, direct community analysis, and RT-PCR. These genotypic methods should be used in conjunction with conventional cultural techniques to improve our knowledge of the gut flora and its interactions. Some techniques are qualitative and give an overall picture of diversity; others are quantitative but require a prior knowledge of the target organisms. Again, a multiplicity of approaches with recognition of technique-specific limitations is needed. Intestinal microbiology must encompass findings generated by these new research tools, although our understanding of major important genera and metabolisms has not significantly changed to date. More pressing may be a need to understand what factors lead to the preservation of numerous related species and strains within some genera.

Genomic approaches have facilitated improved probe design (in some cases to the species or strain levels) and are being increasingly applied to both probiotic and prebiotic research. One fundamental observation is that there are age related changes in the gut microflora composition. Moreover, there may be geographical variation and less commonality than previously perceived between individuals.

More than 500 microbial species are believed to occupy the human gastrointestinal tract and this composition remains largely unknown and highly variable within different locations and among different individuals. The microbial content of the small and large intestine is not adequately reflected by fecal analysis,[54] which has been the predominant sample analyzed to date. The application of PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), Terminal Restriction Fragment Length Polymorphisms (TRF), and high-throughput sequencing of 16S rRNA libraries to the study of the microbial ecology of the gastrointestinal tract has begun to identify the major culturable and non-culturable populations, and it provides the means to study changes over time and under different conditions.[55–59] Fluorescent in situ hybridization (FISH) in combination with flow cytometry is also facilitating high-throughput enumeration of groups within the microbiota.[60] Activity as measured through mRNA transcriptomics and metabolomics in concert with NMR spectroscopy could further monitor changes within the gut.[61] A major bottleneck resides in the inability to examine in real time and under normal circumstances, specific sites within the human intestinal tract. At present, colonoscopy or gastroscopy require emptying bowel contents, and therefore removing many of the organisms of interest. New microchip-based technologies that can be swallowed and into which various detectors could be placed, might provide a means to undertake these studies in the future.

Methods have now become available for whole genome amplification of uncultured cells (lower limit of approximately 1000 cells) where the functionality of 60% of the genes in the genome can be predicted through sequence analysis.[62] As a result of these genetic approaches, our view of the microbial composition of the human gut and other sites such as the vagina[63] will be expanded considerably in ensuing years, particularly in cataloging the collection of unculturable organisms occupying mucosal tissues. These approaches will undoubtedly contribute vastly to understanding the taxonomy of gut and mucosal microbes, and provide a more complete database from which one can measure the impact of probiotics and prebiotics to alter, protect, or re-establish that collective flora. As the list of newly discovered commensal organisms constituting the normal microflora continues to grow, it is also anticipated that new probiotic candidates will be revealed[63] as well as prebiotics with multiple functionality.

APPLICATIONS TO CANCER

Some activities of the intestinal flora have been hypothesized to increase the risk of colon cancer.[64] In as much as probiotics and prebiotics may alter these activities, they may play a role in reducing this risk. Mechanisms of inhibition of mutagenicity have been studied and evidence suggests the following: (1) binding of mutagens (such as heterocyclic amines IQ, MeIQ, PhIP, Trp-P-2, Glu-P-1, Aflatoxin B1 and benzo(a)pyrene) by probiotic strains; (2) degradation of

mutagenic substances such as genotoxins and tumor promoters such as β-glucuronidase, α-glucosidase, nitrate reductase, ammonia, (3) repair or prevention of DNA damage, (4) increased activity of enzymes or processes that protect cells against carcinogen induced damage, such as glutathione transferase (induced by *B. longum* combined with lactulose and resistant starch), hepatic uridine diphosphoglucuronyl transferase, colonic NADPH-cytochrome P450 reductase (induced by various LAB), and enhanced removal of O^6-methylguanine from colonic mucosa; (5) increased apoptosis in the distal colon where LAB inhibit AOM-induced cell hyperproliferation and ornithine decarboxylase activity; and (6) other mechanisms, which could involve fermentation products (eg, butyrate and lactate), bioactive components (eg, peptides, nisin, and bacteriocins) produced in fermented milks, increased mucus production or changes in the mucus profile, calcium activity on epithelial mucosa and in the lumen, or decrease in gut transit time.[53,65]

Clearly, further studies are required with respect to the anti-cancer effects of probiotics and prebiotics in the gut. Clinical studies should compare probiotic strains or prebiotics with chemotherapeutic or other standard medical therapy, wherever possible. Four main groupings of biomarkers should be included in any future clinical trials: (1) cancer end points such as adenoma recurrence; (2) tissue markers such as mucosal cell proliferation, apoptosis, DNA adducts, DNA damage, DNA repair, oncogene/suppressor gene mutations, Cox-2 gene expression, GST activity/expression, cytochrome 450 activity, sialomucins/sulphomucins, genomics (microarrays, RT PCR), and proteomics; (3) fecal markers such as enzymes (glucuronidase etc), ammonia, N-nitrosocompounds, DAG, secondary bile acids, calprotectin; and (5) fecal markers such as cytotoxicity, genotoxicity, apoptosis, AP-1 gene transcription, and COX-2 induction.

Animal studies could elucidate at what stage protective effects can occur and how therapy can be optimized. The production of carcinogens, such as nitrosamines, by organisms in the gut can result in cancer at distant sites. Thus, patient selection need not be confined to colorectal cancer, and indeed some studies have suggested that oral probiotics can reduce the recurrence of bladder tumors.[66] Unlike chemotherapies such as cis-platin, probiotic effects are likely to take place over a much longer timeframe. Such long-term studies have not been undertaken to date. Consideration should be given to genetic susceptibility of people, such as family history or conjoint disease such as colitis.

EXTRA-INTESTINAL SITES

There is mounting evidence to suggest that the action of probiotics is not limited to intestinal activity. These additional targets for action include:

- Prevention of vaginal infections including bacterial vaginosis (BV) and yeast vaginitis
- Prevention of urinary tract infections (UTI)
- Inhibition of the growth or activity of *H. pylori* in the stomach
- Alleviation of kidney stones
- Reduction of infections of the nasopharanyx
- Reduced incidence of dental caries

As with other probiotic research, the proposition of probable mechanisms of action followed by evaluation of these mechanisms is important to progress of the field. Although this is a diverse group of targets, they are bound together by the fact that microbes play a role in the pathology. With this in mind the following investigations are applicable:

- Determine if antimicrobial substances such as bacteriocins, hydrogen peroxide, protein biosurfactants, or cell signals that are produced in vitro are actually produced in situ, and thereby lead to pathogen inhibition or restoration of a normal flora and concomitant improved well-being or reduced risk of disease;
- Verification of a reduction in virulence expression by pathogens in the presence of probiotic strains (and/or their fortification through prebiotics) at the site of action and within the context of biofilms where appropriate;
- Determine the composition of the normal flora of the target region using molecular tools such as DGGE or terminal restriction fragment length polymorphism (TRF), and show how prebiotics and probiotics can impact this flora with respect to age (birth to elderly), genetic background, gender, diet, and hormonal changes (eg, menstrual cycle);
- Identify and characterize receptor sites for probiotic organisms at site of action and correlate receptor density with genetic profile, such as presence of genes predisposing to BV or UTI;
- Determine the impact and implications of prebiotic and probiotic use on the host innate and acquired immune response, for example impact on the cascade leading to preterm birth or destruction of sIgA by gram negative anaerobes.

Clinical evidence is growing, which indicates a strong correlation between absence or disruption of the indigenous microflora at various colonized sites and the onset of diseases.[67,68] This has led to use of normal flora to prevent infection in non-intestinal sites. For example, the "normal" flora in the nasopharynx of a neonate in the intensive care unit includes alpha hemolytic streptococci as a predominant organism.[69] This observation led to the implantation of 1 of these strains into 22 infants leading to colonization and no episodes of infection in a population at high risk for such problems.[70]

The bacterial interference concept was further examined by Saigh et al[71] in relation to sexually transmitted bacterial disease. The conclusion from a study of the vaginal micro-

flora of 229 women was that women with fewer infections caused by gonococci also harbored lactobacilli that inhibited growth of the *Neisseria gonorrhoeae* pathogen ($P < 0.05$). Such protective aspects of lactobacilli in the vagina require continued study.

These lines of investigation have emphasized the important role of the colonizing flora in the prevention of disease and the potential role of intentionally administered microbes to enhance the activity of the normal flora in extra-intestinal sites.

CLINICAL TRIALS

When preparing human trials to assess the impact of probiotics and prebiotics on clinical endpoints, there are several important criteria to consider: subject selection and randomization; the timing of any environmental/cluster effects (eg, outbreaks of infections in a hospital ward containing study subjects); and recruitment of proper subjects (e.g., with or without a history of disease recurrence, healthy or hospitalized, pregnant, non pregnant or postmenopausal women). A comparative group (placebo or control) should be designed to minimize any "placebo" effect and provide meaningful comparisons to determine the impact of the probiotic or prebiotic on the health of the recipient. Dosage must also be determined and should be based on the minimal amount required to obtain a detectable effect up to a maximum that has few or no adverse effects but optimal benefit (risk-benefit ratio). The latter also takes into account selection of best duration of administration.

Outcomes for a clinical study optimally focus on primary endpoints such as the ability to prevent, treat, and reduce or delay disease episodes. In addition, secondary outcomes are valuable, and they usually involve testing a hypothesis for mechanistic effects such as the impact on viral shedding or pathogen virulence. An outcome for a healthy group might include delay of an adverse effect in a genetically prone population. In addition, outcomes can include standard reputable quality of life measurements, such as health related quality of life (HRQL) scores using the individual domains and composite physical and mental health scores of the SF-36 Health Survey.[72]

Follow-up studies are critical to determine whether the disease returns or becomes worse on cessation of probiotic or prebiotic use: Is a newborn baby's health improved because of probiotic or prebiotic use by the mother (allergy, necrotizing enterocolitis, and UTI)? How long does the probiotic organism remain in the baby's gut? If the probiotic becomes permanently established in the baby, what effect does this have on his/her health? What are the medium-to-long-term effects? Any change that deviates from pretreatment and that has negative impact on measurable host parameters should be regarded as an adverse event. Local and systemic effects should be examined. Symptoms, signs, and relevant physiological indicators should be noted, including changes in immune function and blood, liver or kidney functions with respect to the normal range.

It is not yet known how much impact genetic background, gender, diet, and environmental stress have on the gut flora composition and activities. Thus, careful epidemiologic and biostatistic studies should ideally precede any trial design. In addition, considering the volume of data that will be acquired, particularly since genetic tools can detect and quantify very low (<100 cfu) levels of microbes, and protein and mRNA chips measure changes down to the nanoscale level, it is essential that bioinformatics be applied. By combining population health and bioinformatics with traditional expertise in research sciences and clinical medicine, our understanding of the role that the intestinal flora, probiotics, and prebiotics have on health, quality of life, and longevity will be enhanced greatly.

THE FUTURE

While methodologies become more complex, it is important to not lose sight of the need to continue to ask simple questions and not disregard simple answers. Scientific initiatives, some of which have been discussed here, will demonstrate that the scientists who participated in ISAPP's inauguration and many others who work diligently in this area, are truly seeking to change the paradigm of healthcare to one that focuses more on health than disease.

Advances in vaccines, nutrition, and cell–cell communication will form components of this new paradigm. The use of LAB as delivery vehicles for biologic compounds has been considered and actively investigated for a number of years.[73–75] Successful examples of metabolic engineering[76] and production of antigens, allergens, cytokines, or single-chain antibodies,[50,73,77,78] have already been reported. Probiotic cultures may offer advantages for enhanced delivery of biologics to specific locations in the gut, mouth, vagina, or other selected tissues. Genomic information and genetic tools continue to be critically important in furthering the development of these applications, and provide opportunities such as tailored gene expression for targeted and regulated delivery of specific biologic compounds. For prebiotics, there is a need for more hypothesis-driven human intervention trials to complement investigative studies designed to ascertain prebiotic activities. Several hypotheses that should be tested include:

- Prebiotic carbohydrate will display a molecular weight optimum, representing a compromise between persistence and selectivity;
- The knowledge of specific oligosaccharide transport and metabolic systems by probiotic bacteria will facilitate the isolation and development of carbohydrate compounds with prebiotic activity;
- A given prebiotic will always stimulate the growth of a specific population of *Bifidobacterium* spp. and of *Lac-*

tobacillus spp. in a human host if other ecological factors are not limiting;
- Isogenic mutants defective in the ability to use a given prebiotic will not be stimulated by prebiotics in mixed culture fermentations or in animal hosts;
- The health-promoting outcome of a probiotic can be regulated by the prebiotic supplied;
- Prebiotics in animal feeds can be used to reduce gas production in livestock or for several other health targets.

An exciting area of research that will greatly influence the future of probiotic and prebiotic therapies is cell–cell communication. It is clear that microbes communicate with the host.[14,53] and with other microbes.[79,80] Since messages can cross between bacterial species, it is possible that probiotic organisms may "persuade" pathogens not to infect the host. By isolating and sequencing these messages, it could be possible to use them as prebiotic-like compounds or for expression of corresponding genes by probiotic strains to reduce the risk of disease onset. Such investigations will require the application of novel proteomic tools,[81] and will use genetic diversity studies[82] to better understand events taking place within a given microbial milieu.

As more controlled studies are carried out with probiotics so that physiological benefits and mechanisms are better understood, probiotic therapy (or intervention in the case of non-disease states) will become more sophisticated with products targeted for specific effects, comprised of as many or as few genera, species, and strains as is needed for the effect, and which are compatible with host genetic determinants. Similar developments are expected for prebiotics as advances in carbohydrate chemistry, microbial physiology, and biochemical engineering will offer opportunities for rational development of novel and effective compounds. Studies on enzymatic systems underlying the degradation of large polysaccharides and transport and metabolism of oligosaccharides within commensals, as well as identification of the genes encoding such pathways, will further advance this important field.

It can be argued that nutrition has done significantly more to improve the quality and duration of life during this past century than surgery and pharmaceuticals combined. Building on this established importance of nutrition in health, it is time for health professionals to take into consideration the role of the human microflora in the maintenance of health and the prevention of disease, and the role that the consumption of probiotics and prebiotics may play in this process. ISAPP recognizes that scientific establishment of the 100-year-old concept of probiotics and prebiotics will require complex and multidisciplinary investigative strategies that integrate microbiology, ecology, immunology, cell biology, genomics, bioinformatics, food science, and medicine. We look forward to combining these disciplines to scientifically advance a new paradigm for probiotics and prebiotics in the maintenance of health and prevention of disease.

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