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MiniReview

Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: An overview of enabling science and potential applications

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Abstract

The application of probiotics and prebiotics to the manipulation of the microbial ecology of the human colon has recently seen many scientific advances. The sequencing of probiotic genomes is providing a wealth of new information on the biology of these microorganisms. In addition, we are learning more about the interactions of probiotics with human cells and with pathogenic bacteria. An alternative means of modulating the colonic microbial community is by the use of prebiotic oligosaccharides. Increasing knowledge of the metabolism of prebiotics by probiotics is allowing us to consider specifically targeting such dietary intervention tools at specific population groups and specific disease states.

Keywords: Probiotics; Prebiotics; Synbiotics; Colonic microbiota

1. Introduction

In the industrialised world, many infectious diseases are treatable and no longer the predominant cause of premature morbidity. Nevertheless, diseases associated with microorganisms are far from resolved by current therapeutics and the discovery of new and antibiotic-resistant pathogens further justifies the search for new strategies to control them [1]. This is exacerbated by the continuous emergence of novel variants of established pathogens. On a chronic basis, inflammatory bowel disease, colon cancer and irritable bowel syndrome have all been linked to the colonic microbial community and its activities. The human gut is a relatively under-explored ecosystem and yet affords the best opportunity for developing interventions to cope with a variety of alimentary canal and genitor-urinary tract diseases through dietary intervention strategies.

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One approach to health maintenance and disease control is the use of dietary bacterial and carbohydrate supplements that aid the host's indigenous bacterial communities form a barrier against invading pathogens. This comprises use of probiotics (''Live microorganisms, which when administered in adequate amounts confer a health benefit on the host'', [2]) and prebiotics (''A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria that can improve the host health'', [3]).

2. The science of probiotics and prebiotics

Recent strides made in the study of probiotics and prebiotics have been made possible with improved understanding of the diversity and function of the human microbiota, including the genomic sequencing of some probiotic organisms. The oral, nasopharyngeal, stomach, intestinal and vaginal ecosystems are clearly very complex. Many currently non-culturable organisms could form an important part of the host's defense [4], while others may be responsible for chronic diseases [5].

2.1. Probiotic genomics

Genomic sequencing of *Bifidobacterium longum* [6], *Lactobacillus plantarum* WCFS1 [7], *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus casei*, *Streptococcus thermophilus*, *Lactobacillus johnsonii* [8], *Lactobacillus bulgaricus* and *Lactobacillus rhamnosus* (underway) provide insight into the potential activities of commensal bacteria, as perhaps best exemplified by the finding of bifidobacterium genes for oligosaccharide utilization [6]. Translational functional genomic studies provide extended appreciation for genes that actually function in vivo [9], including those involved in housekeeping functions, metabolism, transport, regulatory systems, stress responses and production of surface proteins, bacteriocins and other antimicrobial agents. Of particular significance is that 30–40% of the determined genes have unknown function. Genes encoding fimbriae-like structures in *B. longum* and in *L. plantarum*, *L. johnsonii*, and *L. gasseri*, production of bacteriocins and extracellular polysaccharides, unique carbohydrate utilization pathways, cell surface proteins/antigens, and mucus-binding proteins have been found ([6–8]; http://www.jgi.doe.gov). Notably, diverse prophage and prophage remnants are common among probiotics suggesting that lysogenic conversion may be an important source of genetic diversity [10].

A few putative genes, such as those associated with antibiotic resistance have been found in some probiotic strains that have been characterized as ''generally recognized as safe'' (GRAS) for human consumption [11]. For example, the *L. plantarum* WCFS1 genome contains four homologs to genes encoding tetracycline resistance; three open reading frames (ORFs) annotating as efflux pumps and a fourth as a ribosomal binding protein [7]. Future studies are now ongoing to discover if such genes are actually expressed in vivo and if they are located on mobile genetic elements, such as conjugal plasmids, transposons, phages, or IS-elements (insertion sequence elements). If so, recombinant probiotic strains could be engineered to delete or inactivate these genes.

2.2. Improving our understanding of the mechanisms of action of probiotics

2.2.1. Production of antimicrobial agents

Many probiotic strains can produce one or more antimicrobial substances in vitro, including hydrogen peroxide, organic acids, diacetyl, bacteriocins or bacteriocin-like molecules [12]. However, as yet none of these have been proven to be key components in health maintenance in vivo. If permitted by ethics review boards, the use of wild type versus gene knock out strains with detection of specific antimicrobial substances in situ could address this question. Additional studies are needed to determine the effect of probiotic organisms on host angiogenin and defensin production.

2.2.2. Blocking of adhesion of pathogens and toxins

It is well established that probiotic bacteria can inhibit adhesion of certain pathogenic bacteria such as *Escherichia coli* and *Salmonella enterica* serotype Typhimurium to epithelial cells in vitro [13]. It is not currently known by which mechanism this inhibition occurs, although competitive binding to receptors or stimulation of host factors such as mucin production have been proposed [14,15]. Similarly, prebiotic oligosaccharides may block common receptor sites for gut pathogens, through their presence in the lumen.

2.2.3. Modulation of the immune response

Administration of probiotic strains causes a range of non-specific and specific host immune responses in diseased and healthy subjects [16]. These include, for example, the enhancement of phagocytic activity of peripheral blood leukocytes and natural killer cell activity. Additionally, stimulation of both non-specific secretory IgA and specific antibody responses, especially mucosal IgA, to mucosal vaccines such as rotavirus, polio and *Salmonella typhi* and enteric pathogens such as rotavirus has been seen [16]. Increased cytokine production in vivo (IFN-γ, IFN-α, IL-2) and by peripheral blood mononuclear cells ex vivo (IL-1β, TNF-α, IL-6, IL-10, IFN-α, IFN-γ) have been reported following appropriate probiotic stimulation [16]. The question is can the reactions be predicted for a given subject, and can they be effectively directed? Few studies have

examined the anti-infection effects and host immune responses in the same subjects following administration of probiotics. Further studies in animals and humans are essential to elucidate the role of probiotics-stimulated immunological mechanisms in protection against enteric pathogens.

The gut barrier function, which protects against the constant exposure to foreign antigens from food and the environment, can be stabilised by probiotic administration. This is thought to arise from stimulation of production of secretory IgA [17] and mucus [14] and by attenuating pro-inflammatory responses [18] such as IL-8, MCP1, MIP1 and RANTES, pro-inflammatory cytokines (TNF-α, GM-CSF, IL-α and IL-1β) and prostaglandins and leukotrienes induced by pathogens.

Probiotics have been administered safely to individuals with immuno-inflammatory disorders such as atopy [19] and Crohn's disease [17] as well as those with HIV and immunosuppression [20]. Treatment resulted in down-regulation of the over-expressed immuno-inflammatory responses by stimulating regulatory T cells, attenuating pro-inflammatory responses and stabilising the gut mucosal barrier [21].

2.3. Improving our understanding of the mechanism of action of prebiotics

2.3.1. Metabolism of prebiotics by indigenous probiotics

Our knowledge of the diversity of the human gut is increasing steadily, largely as a result of improved DNA-based methods of probing complex bacterial ecosystems [22–25]. This increased knowledge will have important implications for studies on the efficacy of prebiotic oligosaccharides and on the development of novel forms with specific functional enhancements. The traditional targets for prebiotics are *Bifidobacterium* spp. and *Lactobacillus* spp. [3]. It is, however, desirable to increase the generation of butyrate in the colon [26] and this is opening up the potential of targeting non-clostridial butyrate producers in the gut [27]. It is certain that new targets will be identified in future. It is also worth remembering that our in vivo data is generally derived from analysis of faecal samples from human volunteers. This provides little data on the details of the microbial community changes occurring in the higher regions of the colon.

The mechanisms by which prebiotic oligosaccharides are selectively metabolised by beneficial members of the gut microbiota are not adequately understood at the present time. There are two general paradigms of prebiotic metabolism. The most documented of which is the possession, by probiotic microorganisms of cell-associated exo-glycosidases [28]. Such enzymes act by hydrolysis of monosaccharides from the non-reducing end of the oligosaccharide, which are then taken up by the cell. This mechanism has been shown to operate in *Bifidobacterium infantis* [28], which possesses cell-associated β-fructofuranosidase activity. An alternative mechanism is the uptake of intact oligosaccharides by probiotic organisms followed by intracellular metabolism, and there is some evidence that this mechanism may operate in some species [29,30].

The extent to which these two mechanisms operate in vivo remains to be shown. This is an important area of study, as this information would facilitate the design of prebiotics with a much higher degree of selectivity, with intracellular metabolism presumably preferred to minimise secondary fermentation by non-probiotic species.

With increasing efforts to expand the range of prebiotics available to the food and healthcare industries, and to develop more efficacious forms, there is a need to quantitatively compare prebiotics. A basic prebiotic index (PI) has been formulated to quantify effects, as based upon in vitro fermentation profiles [31]. It provides a comparative quantitative index that increases if desirable bacterial groups (currently bifidobacteria and lactobacilli) increase and/or undesirable groups (currently bacteroides and clostridia) decrease. One problem with this quantitative approach is the variability of the gut microbial community among individuals. A way to obtain a more reliable comparative assessment might be to use standardised, lyophilised bacterial mixtures to quantify prebiotic action. There is, however, a danger of oversimplifying a complex phenomenon and of missing subtle effects on the colonic microbial community. All such in vitro comparisons must be substantiated in human volunteer trials if the strengths and weakness of particular prebiotics are to be identified.

2.3.2. Antimicrobial oligosaccharides

Prebiotics induce antimicrobial effects principally via their selective stimulation of indigenous beneficial strains, which secrete antimicrobial compounds, modulate immune function and compete with pathogens for receptors. However, the potential exists for soluble oligosaccharides (say incorporated into foods) to be used as a means to competitively bind pathogens and reduce their ability to colonize and infect the host [32]. In light of the numerous ways by which some pathogens can colonize, the specificity and effectiveness of prebiotic receptors needs to be tested thoroughly. Balancing benefits with risks, such prebiotic therapy could be a useful adjunct for vulnerable subjects such as the elderly, formula-fed low birth weight infants, persons taking antimicrobials and travellers to developing countries.

Some prebiotics such as chito-oligosaccharides have direct antimicrobial activity, preventing bacterial growth [33]. The activity is size-dependent with short oligosaccharides being most effective [34]. The species-activity spectrum of chito-oligosaccharides is not known with any certainty, nor is the degree of antimicrobial

activity exhibited in the human gut. This is an important area for study as chito-oligosaccharides are currently sold as fat-blocking slimming aids.

2.4. Developing prebiotics for specific probiotic strains

Prebiotic structure, including chain length, branching, linkage types and the presence of mixtures of different molecules can affect the fermentation specificity of these compounds [35,36]. As such, small molecular differences in prebiotic structure may induce significant changes in physiological functions. For example, many fructo-oligosaccharide products are available and it is apparent that products with higher molecular weight may be more slowly fermented and thus persist for longer in the colon. Combinations of inulin (DP 10–65) and oligofructose (DP 2–8) may elicit synergistic effects. If it were possible to match prebiotics with probiotic strains, the physiological benefits may be enhanced [37]. Alternatively, probiotic strains might be selected for their ability to generate prebiotic oligosaccharides, which are then preferentially utilized by the producing probiotics [38–40].

2.5. Intervention studies to substantiate claims

Health and functional claims for pro- and pre-biotics are based on a wide variety of studies, including in vitro experiments, animal models and epidemiology studies. However, in order to substantiate fully such claims there is an absolute need for human trials – these may be observational or more usually intervention studies. The design and conduct of such trials in healthy adults are well described, but use of pro- and pre-biotics is increasingly being targeted towards specific groups in the population, notably the very young and the elderly. Studies in such population subgroups create additional challenges for intervention studies.

2.5.1. Interventions designed for newborns and infants

It is well documented that breast-fed infants have a colonic microbial community more dominated by bifidobacteria than do their formula-fed counterparts, who harbour a more complex and adult-like microbial community co-dominated by bifidobacteria, bacteroides and to a lesser extent clostridia [41]. Although diet alone does not determine microbial community development, examination of the bifidobacterial composition of breast- and formula-fed infants, at the sub-species level, demonstrated that distinctive biotypes are harboured by each infant [42]. It has been postulated that the infant microbial community provides a blueprint for gut function and adaptation throughout life and thus may impact disease development and dysfunctions including allergy, autism, asthma and gastrointestinal disorders [43].

Most intervention studies in infants to date relate to probiotics, although data are accumulating on prebiotic and/or synbiotic products [44]. Probiotics have been shown to reduce the incidence of diarrhoeal, respiratory and caries infections in children [45], atopic dermatitis in babies [46] and necrotizing enterocolitis in pre-term newborns [47]. Probiotic administration during weaning can alleviate some of the common symptoms associated with transition to a more complex diet, including reduced incidence of acute diarrhoea, reduced constipation and reduced food intolerance because of down regulation of the inflammatory response [48]. Additionally, certain probiotics and prebiotics may enhance calcium absorption and clinical trials in children have shown improved bone density levels [49].

Several issues need to be addressed in order to perform good quality trials in children. The differences, if any, between the inherited microbiota of vaginal versus caesarean babies needs to be determined, especially given the increased rate of caesarean births. Likewise, differences arising from feeding practices (breast or formula) and introduction of various types of weaning foods, often influenced by social, cultural and environment factors, need to be better understood.

2.5.2. Therapeutic interventions for the elderly

With advancing years the gut microbial community changes to a more diverse composition [50]. It is generally accepted that bifidobacterial levels markedly decrease after 55–60 years of age [51], for reasons unlikely to be explained by changes in diet or hormones (as the microbial community of men and women alter), but perhaps associated somehow with immunological, physiological and/or lifestyle factors. Such microbiota changes could render subjects more susceptible to gastrointestinal problems, or to diseases associated with bacteria in the gut (for example cancers, arthritic or allergic diseases). Functional foods may have a particular application in this high-risk group, especially in terms of protection against entero- and urogenital pathogens. Products containing probiotic bacteria isolated from the elderly (as probiotics or synbiotics) are under development for elderly subjects [52].

There is a need for long-term studies to map the microbial community of the elderly, and investigate relevant biomarkers, specific diseases and bowel functionality with respect to ageing [53]. Ideally, studies should be standardised to allow comparison between laboratories, study groups and clinical trials. It is also important to identify outputs (clinical and otherwise) to enable the greatest information output from such trials. For the elderly, definitions are problematic for example in distinguishing between ‘younger’ and ‘older’ seniors. The threshold between these groups is arbitrary and may be defined either by age, by health status or by level of independence. Also, elderly people are more likely to

be receiving medication for various ailments, but may be otherwise regarded as healthy. These issues as well as levels of sensory and cognitive impairment need to be considered.

2.6. Application of probiotics in developing countries

In countries such as India, the overuse of antibiotics and poor nutrition in children, together with inadequate treatment for diarrhoea has created a major health problem [54]. Many families use fermented milk (curd or Dahi) as part of their daily diet, sub-culturing the organisms themselves. There is some evidence of an improvement in recovery from diarrhoea, but the effects appear to be heightened by addition of probiotic bacteria [55,56]. One particular strain, *Lactobacillus casei* DN-114001, has been reported to reduce diarrhoeal morbidity by 40% in children [56]. While this suggests strongly that probiotic interventions have potential to benefit many people in India, it must be considered that the population prefers local solutions to health problems and is unlikely to regularly purchase a commercial milk product containing a probiotic. In addition, the environmental conditions in India are not conducive to maintenance of probiotic viability, and very few companies have developed formulations with good shelf-life at 40–48 °C.

In Sub-Saharan Africa, HIV is spreading rapidly. The region has only 10% of the world's population but 70% (29.4 million) of HIV infected people. One in three children entering hospital has HIV and over 1 million children are orphans because of the disease. UNAIDS reported in 2002 that women account for 58% of HIV/AIDS cases and this is growing. Studies in Africa and Asia have shown that one major risk factor exists for HIV, gonorrhoea and chlamydia acquisition in women: namely the absence or depletion of lactobacilli in the vagina associated with an overgrowth of anaerobic pathogens causing bacterial vaginosis [57,58]. A displacement of lactobacilli, for example by *Gardnerella* spp., elevates the vaginal pH and creates an environment within which the pathogens survive and can infect the host. The risk of HIV is doubled when the woman has bacterial vaginosis compared to a lactobacilli-dominated normal vaginal microbial community [59]. The potential exists, therefore for the modification of the vaginal microbial community by probiotic intervention to prevent the consequent infection with HIV. The critical component in treatment or prevention of bacterial vaginosis with probiotics is for the applied organisms to colonize the vagina. Studies have shown that *L. rhamnosus* GR-1 and *L. fermentum* RC-14, able to kill viruses within minutes, administered intravaginaly or orally, can colonize the vagina for several weeks without any adverse effects [59,60]. It would seem that such an approach to HIV management is worthy of much closer attention.

3. Potential barriers to success

3.1. Scientific barriers

One of the biggest barriers to more widespread acceptance of probiotic and prebiotic concepts amongst the scientific and medical communities (academic and otherwise) is the limited appreciation for the role that commensal microbes have in the human body. Improvements in culturing techniques are needed to isolate many inhabitants of our body, allowing better functional studies to take place. Such an understanding is essential to empower rational ecological intervention to improve host health.

We need to know much more about the cross-talk [61] amongst the bacteria and between organisms and human cells. In doing so it may be more feasible to 'program' humans at birth to receive a microbiota best able to maintain their life-long health.

Experimental standards used in the field must be standardized so that bacterial growth simulates the in vivo situation, and human studies use adequate sample sizes, well-selected subjects and clinically important outcomes [62]. In addition, the genetic stability of probiotic strains needs to be monitored, especially in populations who have consumed strains for months and years.

Such combined efforts, along with integration of the concepts of probiotics and prebiotics into school, college and university curricula, and healthcare practise, will lead to widespread acceptance and a better understanding of the benefits and limitations of these concepts.

3.2. Economical considerations

Most of the successful prebiotics in the world market are derived from waste materials or cheap agricultural produce [63]. An economical source of novel prebiotic oligosaccharides includes waste biomass [64], large quantities of which are produced in food processing operations around the world. Extracellular polysaccharides elaborated by lactic acid bacteria are also a promising source of prebiotics [65]. Extracellular polysaccharides from *Pediococcus* spp., *L. plantarum* and *L. sanfransiscensis* have been found to be bifidogenic and not to be utilised by *Clostridium perfringens* or *E. coli* O157:H7. Recent developments in glycotechnology could result in enzymatic manufacturing of oligosaccharides of biological importance [66].

In terms of probiotics, lactobacilli and bifidobacteria are not easy to grow and retain in suitably high viable counts, especially the latter and there is a need for clear quality assurance criteria for probiotic bacteria in food products [67]. Some products have clearly not taken this into account, perhaps explaining the existence of overly cheap retail products containing dead organisms or contaminants. There needs to be a balance between

manufacturing technologies that are economic for consumers but sufficient to deliver shelf-stable products able to deliver the best dose to a specific body site [68].

3.3. Regulatory barriers

The biggest barrier to commercialisation of new probiotics and prebiotics is the regulatory process. There are major differences in regulations in the EU, Japan, Canada and the USA and getting a new product through this process is a tedious, slow and increasingly expensive undertaking [69]. Failure of countries to implement logical, science-based guidelines, such as those prepared by the Food and Agriculture Organization of the United Nations and World Health Organization (2001), is severely impeding progress and the availability of products of potential benefit to millions of people.

A further obstacle is the inability to make meaningful health claims for new products even with substantial scientific evidence [70]. Unfortunately, the ''drug versus food'' labelling standards of old have proven hard to change. Such changes are further delayed by companies, which produce vague and misleading labels or use species inside their product that either do not exist (e.g., *L. sporogenes*) or differ from those on the label. There is, however, new Canadian, US and EU legislation on permitted health claims currently under development.

4. Concluding remarks

The rapid and dramatic increase in scientific, medical and lay public interest in probiotics and prebiotics has propelled these areas closer to the mainstream of healthcare and consumer product lines. However, only for a few probiotic strains and prebiotic products has the extent of clinical evidence been extensively generated. The potential certainly exists for targeting of these agents at specific disease states and population groups, but this can only be realised by the generation of clinical or consumer documentation, adherence to strict guidelines and attainment of high quality assurance product standards.

Modern molecular, nanotechnology and immunological tools must be directed towards more thorough understanding of microbial community structure and function. In turn, this will generate a new level of understanding of how the human body functions with its microbial constituents, and how such interactions can be modulated for the betterment of the host.

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