Human gut microbiota and future prebiotics

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Rapid advances in molecular methods that enable culture-independent analysis of the complex bacterial populations is increasing awareness and understanding of the composition and activity of the microbiota in the human gastrointestinal tract, its role in host health and response to changes in diet and lifestyle. In this article we discuss the shortcomings of the contemporary approach of targeting a few selected bacteria, notably lactobacilli and bifidobacteria, to gauge the status of the gut microbiota for promoting health of the human host.

The lumen of the gastrointestinal tract contains roughly 10 times as many microbes as there are cells in the human body. Although present along the entire length of the gut, the vast majority of bacteria occur in the colon and the nature of the relationship with the host is largely commensal. Mucosal homeostasis is maintained through frontline intestinal immune defences; however, the colonic microbiota contributes to this barrier function through colonisation resistance/competitive exclusion. The microbiota benefits the host in many other ways, including salvaging energy from dietary components that escape digestion and absorption in the upper gut and would otherwise be lost to the faecal stream (for example, fibre), and by producing vitamins and metabolites important to host health[1,2]. The discovery of alterations in the populations of the gut microbiota in individuals with gastrointestinal disorders such as inflammatory bowel disease[3] is generating greater awareness of the role of microbes in human health, and with it strategies that can be employed to prevent or correct microbial dysbioses. The composition of the gut microbiota is influenced heavily by the physicochemical properties of the luminal environment, which in turn is determined largely by the diet of the host. Hence, one strategy employed to influence health via the microbiota is through dietary intervention. An alternative, and more established, strategy is to deliver live, potentially beneficial microbes to the gut. These two strategies form the basis of the prebiotic and probiotic concepts respectively. The practical focus of these approaches to date has been to bolster existing populations of lactobacilli or bifidobacteria in the large bowel, or to deliver new populations of bacteria belonging to these genera to that site. Bacteria belonging to these genera are considered beneficial to adult health, but the evidence from many investigations to support the claim that these bacteria are primary mediators of host health is underwhelming. The question then is which gut microbes should we be turning our attention to? To answer this question we will discuss the likely origins of the present focus on lactobacilli and bifidobacteria in prebiosis and probiosis and then describe emerging knowledge of microbes important for gastrointestinal and systemic health and wellbeing of adult humans.

Faecal bifidobacteria and lactobacilli in infants

The gut is sterile until colonised with microbes during birth or shortly thereafter. The consequences of caesarean birth as opposed to vaginal delivery on microbial colonisation are not fully understood but infants born naturally have higher faecal bacteria counts at one month of age[4]. Of perhaps greater importance to the development of a robust gut microbiota and long-term health of the individual is whether the infant is breast-fed or formula-fed. The former may protect against a range of conditions in later life (for example, eczema and asthma) which may be partly due to the presence of oligosaccharides that stimulate the growth of bifidobacteria and less numerous bacteria such as lactobacilli, which, in turn, promote the development of normal immune functions[5-7]. The infant gut may also derive bacteria from breast milk, although not all studies are equivocal[8,9]. Over a century ago Mechnikov proposed the

use of fermented milk products to stimulate growth of beneficial bacteria and also promoted the idea of improving gut health and increasing longevity by ingesting probiotic bacteria[10]. It is, therefore, not surprising that the majority of bacteria used as probiotics belong to these genera as they are relatively easy to culture and there is a long-held view that they are of benefit for human health. However, at weaning, infants switch to a more varied diet usually consisting of solid foods, including cereal and cereal-based foods, which contain different types of fibre. The large bowel microbiota is then exposed to many varied forms of fibre, as well as other substrates, for the first time, which results in large shifts in microbiota population diversity and activity. Most notably, the bifidobacteria populations decline in abundance, and like lactobacilli represent only a small fraction of the large bowel microbiota[11]. Consequently, microorganisms other than the lactobacilli and bifidobacteria are likely to contribute substantially to the health of the adult gut.

Probiotics and prebiotics in the context of the adult gut microbiota

The present challenge is to understand what constitutes a healthy gut microbiota in adults and weaned children. This can be achieved in part by understanding the gut microbial populations and activities associated with diets linked to favourable health outcomes. Epidemiological studies indicate that dietary fibre is associated with lower risk of diseases such as colorectal cancer whereas a diet high in red and processed meat is associated with increased risk[12,13].

Studies carried out by us and others have shown that the protective actions of different dietary fibres, including resistant starch, appears related to their ability to undergo fermentation and generate short chain fatty acids (SCFA) within the large bowel, in addition to other well-known actions of dietary fibre, such as faecal bulking, which helps dilute and eliminate toxins. In rodents, inclusion of high levels of resistant starch in the diet protects against colonic DNA damage induced by diets high in protein or red meat, and against carcinogen-induced colorectal tumours[14-19]. That protection is correlated strongly with production of SCFA, especially butyrate. The SCFA, mostly acetate, butyrate and propionate, have many beneficial actions[2,20]. They are the primary energy sources for cells lining the large bowel; they stimulate production of the mucus lining the colon, and butyrate enhances the apoptosis of damaged colonic cells. Acetate also inhibits the activities of some potentially lethal enteropathogenic bacteria and bifidobacteria can contribute to its production[21]. It is, therefore, increasingly apparent that bacteria involved in the production of butyrate, and other SCFA, are important for adult colorectal health and may also have systemic benefits. Conversely, there is speculation that energy derived from colonic fermentation of fibres through SCFA production could contribute to obesity in some individuals but this is yet to be confirmed.

Numerous bacterial species present in the human gut have the capacity to generate butyrate. One of the most widely studied, *Faecalibacterium prausnitzii*, is of great interest because it also has anti-inflammatory actions independent of butyrate[22]. Such bacteria could be the probiotics of the future, but technologies for growth, storage and delivery of a greater range of bacteria need to be developed. But this begs the question, why use probiotics at all, especially in situations where the bacteria being delivered to the gut are already present there? Why not simply consume foods that contain dietary components that selectively stimulate the growth and activity of beneficial bacteria? This concept is known as prebiosis. A recent consensus definition of a prebiotic is a dietary component which leads to "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host"[23]. The most widely adopted interpretation of prebiosis to date has been the ability to selectively increase numbers of faecal bifidobacteria and lactobacilli, no doubt partly due to the reliance on these genera for use as probiotics. As our knowledge of gut physiology and the impacts of the adult microbiota grow it should be possible to more clearly define foods with prebiotic potential.

Individual differences in gut microbiota

Our growing knowledge of the significant variation in the composition of the gut microbiota that occurs between individuals, and within individuals over time as a result of ageing or other influences, is one area which is likely to assist in developing suitable prebiotics. Metagenomic analyses led to the proposal that the human microbiome of each individual is typified by one of three enterotypes, each characterised by different dominant groups of microbes that may be modulated by long-term dietary patterns[24-26]. The most recent and most comprehensive analysis of the human microbiota (through The Human Microbiome Project) has confirmed the presence of unique combinations of microbes in the gut of individuals and the results are in support of enterotypes when stool microbiota are examined at the genus, but not species, level[27,28]. These differences could help explain some of the variation in responses generally seen in human dietary interventions. For instance, this could explain why some people have consistently lower concentrations of faecal butyrate even after the consumption of a diet high in resistant starch[29]. We have shown that *Ruminococcus bromii* is strongly linked to the ability to produce SCFA in response to resistant starch consumption in humans[30] and so individual differences in populations of such bacteria may determine responses to changes in diet. Consequently, the effectiveness of a prebiotic used to improve bowel health through production of butyrate may be dependent on the microbiota profile of an individual. Accordingly, the one-size-fits-all approach for probiotics and prebiotics may not work for all individuals[31]. In the future a deeper knowledge of the microbiota of each individual may be possible and lead to personalised probiosis or prebiosis.

The prebiotic index

How then do we compare the effectiveness of prebiotics in the future? Roberfroid[32] made a start by introducing the prebiotic index, which compares different prebiotics based on their ability to increase the numbers of bifidobacteria and lactobacilli, also

taking into account the dose and the initial populations of these bacteria. However, as argued above, these bacteria are probably not the most important contributors to adult health. We have previously suggested broadening the perspective of prebiotics, without changing the definition, by considering changes in colonic fermentation products such as butyrate as key indicators of bowel health, which would result in acceptance of a greater range of dietary components as prebiotics, including resistant starch[33]. Our laboratory is, therefore, currently seeking to refocus the prebiotic index. It will take a broader range of factors into account, including SCFA production and a wider (and more relevant) spectrum of gut microbes that impact the health of adults and children. This should provide a more relevant and more effective tool for assessing the health-promoting benefits of potential foods or food ingredients.

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Biographies

Dr Michael Conlon is a senior research scientist at CSIRO Animal, Food & Health Sciences in Adelaide, and is the leader of the Gut Biomarkers capability. He is also a leader of projects within the Preventative Health National Research Flagship and the Food Futures National Research Flagship that focus on gastrointestinal health, with a particular emphasis on understanding the health benefits of dietary fibres and the physiological, biochemical and microbiological processes that contribute to colorectal cancer, inflammatory bowel disease and other conditions.

Dr Tony Bird is a principal research scientist at CSIRO Animal, Food and Health Sciences and Research Stream Leader in Food Futures National Research Flagship, specialising in the substantiation of the health benefits of foods and food components. His current research interests include the development and application of a range of methods and techniques, from *in vitro* systems to dietary intervention trials in humans, to investigate the health-promoting properties of carbohydrates and their impact on indices of chronic disease risk.

Dr Claus Christophersen is a research scientist at CSIRO Animal, Food and Health Sciences in Adelaide and responsible for the development of molecular microbial capability and works in Food Futures National Research Flagship projects substantiating the health benefits of new foods and food ingredients. His interests are in understanding the interactions between the microbiome, diet and the host and in developing molecular and biochemical markers of a healthy gut.