Which Bacteria Should We Be Feeding When We Eat Dinner: How and Why?

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Introduction

The human digestive tract, particularly the colon, is one of the most microbially active ecosystems in existence. The gut contains a massive variety of bacteria, viruses and yeasts/fungi. Whilst eukaryotes are present in small numbers only, it has been recently reported that huge numbers of viruses exist, many of which are of undescribed identity. This has raised the possibility that new bacteriophages may be described to combat bacterially induced disorders. However, it is now evident that diet can have a considerable bearing upon the risk of disease induction – through targeting the activities of indigenous bacteria.

Acquisition

Shortly after birth, the previously sterile infant gut begins to be colonised by an array of bacteria. The newborn will first come in contact with bacteria from the birth canal and its surroundings. Factors such as microbial flora of the female genital tract, sanitary conditions, obstetric techniques, vaginal or caesarean mode of delivery and type of feeding all have an effect on the level and frequency of various species colonising the newborn infant gut. Microorganisms are therefore transferred into the hitherto sterile infant gut, with initial colonisers being facultative anaerobes, and therein they find a warm nutritious environment for their growth. The bacteria rapidly remove traces of oxygen present in the gut and the system becomes strictly anaerobic within one week of birth. There are thought to be major differences in the microflora

profiles between breast and formula-fed infants. The former have a predominance of bifidobacteria, a perceived health promoting genus, whilst the latter have a more complex community structure with no one group predominating. Human milk contains a complex mixture of glycoproteins and oligosaccharides that are stimulatory to the bifidobacteria. These, in turn, have powerful inhibitory properties against various gastrointestinal pathogens. This more than likely contributes towards the 'breast is best' hypothesis. What is certain is that bottle fed infants seem to experience higher infection rates than those who are breast fed. As such, significant moves are now under way for formula food manufacturers to alter their product constituents to more effectively stimulate bifidobacteria. New products containing prebiotics (see later) have been recently launched in Asia, Europe and the Americas.

Upon weaning, a more varied diet is ingested and microbial populations respond by becoming much more complex in nature. At about 2 years of ages an 'adult like' composition exists. Here, at least 500 bacterial species exist with probably an equivalent number not yet described.

Bacteria numbers in the human stomach are approximately 10^3 per ml of contents (Fig. 1). This is because a rapid transit time and high level of acidity maintains fairly sterile conditions. One notorious example is *Helicobacter pylori* which resides in the gastric mucosal layer and has been associated with certain clinical states like Type B gastritis, dyspepsia and stomach carcinomas. *H. pylori* is arguably the most researched microorganism in the last two decades. In particular, observations that carriage of a specific pathogen may predispose towards a particular clinical state has raised the whole profile of gut microbiology and the ensuing health consequences.

In the small intestine, microbial numbers can reach up to one million per ml. A short transit time in the ileum and jejunum as well as an input of bile salts and pancreatic enzymes, maintains the flora at this level.

The human adult colon is about 150cm in length with a typical transit time of 24-72h. This is a significant period for bacteria to grow to extremely high levels. Moreover, the supply of growth substrate is plentiful with around 100g of dietary residues (carbohydrates, proteins, amino acids, lipids, etc.) entering the colon daily and

fortifying indigenous sources for growth like mucus secretions, epithelial cells (Fig. 2). Here, bacterial numbers can reach up to 10^{12} per ml of contents (which is probably as many bacteria as can be contained in 1ml), of which several hundred exist in the adult. This microbial mass makes the colon one of the most metabolically active organs in the body and certainly the most heavily colonised. In fact about 90-95% of total cells in the body are thought to be large intestinal bacteria i.e. quantitatively humans are many fold more of microbial composition than mammalian! When considering the types of bacteria that can be fed with dinner, the colon is the principal organ involved. This is given added significance by the impact that gut bacteria can exert in health and disease.

The Role of Gut Flora in Health and Disease

Through its metabolic activities the colonic microbiota can have a significant impact upon host welfare. For example, the principal end products of the anaerobic fermentation are organic acids. Some of these are absorbed from the gut and can be metabolised systemically. Through such a process, it is believed that something like 10% of a person's daily energy requirement can be derived from gut bacteria. It is not inconceivable therefore to conclude that the microbiology of the colon can have an impact upon mood and other markers of well-being. This is supported by the research of Andrew Smith and colleagues at the University of Wales, Cardiff, UK who reports studies whereby the feeding of high fibre (a readily metabolised growth substrate for the gut flora) has positive effects upon energy, stress and cognitive performance. More specific end products of gut bacteria, like propionic acid, are thought to interfere with cholesterol synthesis in the liver and may therefore be useful for protecting against coronary heart disease. Moreover, the gut is the principal immune organ of the body a fact which is at partly attributable to the antigenic status offered by the resident microbiota.

Building upon research with *H. pylori*, several digestive disorders are being researched for their link with specific gut flora components. Some are more speculative than others, but the below are examples of research focus

- Ulcerative colitis (UC): a disorder principally of Western origin which typically onsets in young adults. This one example of an inflammatory bowel disorder (IBD). It is confined to the colon where most bacterial cells in the

body reside. Several lines of evidence have pointed to microorganisms as a causative or maintenance factor, e.g. UC cannot be induced in animal models lacking a gut flora. Our own research and that of others have indicated that sulphate-reducing bacteria have an involvement. Sulphate reducers are ubiquitous in the colitic gut, as compared to healthy persons, and they produce sulphide which is an extremely destructive cellular toxin. Sulphides also interfere with butyric acid oxidation in colonocytes, thereby affecting their function. Our group is currently carrying out a dietary intervention study in UC patients designed to reduce sulphate reducing activity through the use of prebiotics, and thereby maintain remission.

- Crohn's disease: a form of IBD that can affect any area of the alimentary tract. Evidence for a microbial aetiology is more suspect than for UC. However, mycobacteria have been implicated by several groups. The epidemiology of Crohn's disease is about one third that of UC at 2/3 in 100,000.
- Irritable bowel syndrome (IBS): an extremely prevalent disorder often related to stress. However, one indicator of stress is gut dysfunction and vice versa. IBS is estimated to affect up to 20% of persons in certain Western civilisations. Symptoms are diarrhoea, constipation or both with attacks being unpredictable. The yeast *Candida albicans* is involved in recurrent vaginal thrush but has also been suspected as a trigger factor for IBS. At the University of Reading we have isolated a probiotic *Lactobacillus plantarum* with potent anti-candida activity. This is now being trialled in IBS sufferers.
- Bowel cancer: colorectal tumours are the second most prevalent forms of cancer in humans. It is responsible for 1 in 5 fatalities in the USA. Components of the gut flora have the capacity to produce carcinogens and tumour promoters from dietary components. Examples include nitrosamines, heterocyclic amines, ammonia, diacylglycerols, IQ, faecapentenes.
- Antibiotic associated diarrhoea: occurs when homeostasis in the gut is disturbed through the use of non-specific antimicrobials. A classical form is pseudomembranous colitis, which is caused by the proliferation of *Clostridium difficile* within the flora. The normal suppressant effect of gut bacteria against *C. difficile* is compromised allowing the pathogen to elaborate two types of toxin.

- Translocation: this often occurs in relation to trauma, such as intensive surgery. The gut can become ‘leaky’ with bacteria migrating to systemic regions like the liver. Therein, they may produce toxins and have destructive effects as a result.
- Pneumatosis cystoides intestinalis (PCI): this is characterised by gas filled cysts lining the colon. The gas is of microbial origin. Sufferers may have an over activity of gas generating genera such as the clostridia. However, studies have also shown that bacteria capable of metabolising hydrogen (sulphate-reducing bacteria, methanogens, acetogens) are missing in the PCI gut.
- Autistic spectrum disorders (ASD’s): early writings of clinicians like Arbuthnott-Lane and Metchnikoff suggested that toxin generation in the bowel could have influences systemically. For example, products of protein metabolism in the gut include amines which have been linked into clinical states like migraine, schizophrenia. Recent evidence, driven from gastrointestinal symptoms often experienced by autistic persons, has shown a prevalence of clostridia in stools. This genus is recognised as being of negative function. Perhaps the toxins are being absorbed into the bloodstream and impacting elsewhere? Irrespective, a high predominance of clostridia is not especially helpful for digestive health.

The discussion above cites examples of bacteria or other microorganisms that should NOT be fed by dinner. What about those which are more benign and/or even health positive? Can they be fortified to help prevent disorder? If so what is the most reliable mechanism? These are crucial questions that have added relevance given the burden of gut disorder, the lack of useful treatments in many cases and increasing cost of pharmaceutical approaches. Probiotics and prebiotics are dietary mechanisms that serve to ‘improve’ the gut flora composition and decrease the activities of pathogens. This is germ warfare that can have positive consequences for those involved.

Probiotics: adding microorganisms to the gut ecosytem

The most widely used and historic approach towards altering the gut flora composition and activities is through the use of probiotics. Here, live microorganisms are ingested in the anticipation that they reach the gut and interact with the flora to

increase a benign community structure. It is thought that humans have been ingesting probiotics for thousands of years ('soured milks'). Nowadays, many different products exist with the dairy sector (fermented milks, yoghurts, cheeses) being the most popular. Traditional yoghurt is manufactured using the strains *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, neither of which are recognised as probiotics. A probiotic version would have other strains added to it, or used in the fermentation procedure. The most common microorganisms used are lactic acid excreting bacteria such as lactobacilli (e.g. *L. casei, L. acidophilus, L. fermentum, L. johnsonii, L. plantarum, L. rhamnosus*) or bifidobacteria (e.g. *B. longum, B. infantis, B. bifidum*). These products are often labelled as bio-, active, probiotic, bifidus, etc. Other organisms used in probiotic products are some yeasts such as *Saccharomyces*, as well as lactococci, streptococococci. The market also contains Gram negative species like the Nissle *E. coli* strain. Other delivery vehicles for probiotics are fruit juices and lyophilised versions in powders, capsules, tablets, sprays. Probiotics are also common on the farmyard where they are said to reduce the risk of infection, increase yield and feed conversion, improve digestion and lead to improved products (eggs, carcass quality, milk). The mechanisms behind these effects are not fully elucidated but generically are linked towards decreased pathogen load in the gut because of increased probiotic presence. Probiotics have been used for the past 40 years in farm animals. Purchases of probiotics for farm animals in USA have increased five fold during the past decade, with over 50% of dairy producers using probiotics. For human use, the market is even larger with a profit income of several billion euros in Europe. If anything, the situation is even more advanced in Asia, principally Japan.

A survey of the literature reports over 50 published human trials with 'positive' results. Principally these are on intestinal problems like gastroenteritis, IBS. However, there are also observations on reduced urinary tract infections with probiotics. Moreover, chronic conditions like cancer, coronary heart disease and IBD have been addressed. Often, the data are variable and this may be related to strain variability, survival in the products and the ability to influence the competitive gut ecosystem. However, given the encouraging data it seems that alteration of the gut flora composition has promise to prophylactically, or perhaps therapeutically, address gut mediated conditions – more specifically those with a microbial element. More

hypothesis driven research on probiotics and the use of up to date methodologies will further determine the realistic health outcomes.

Prebiotics: altering the composition of the indigenous gut ecosystem

Many different factors like age, stress, antimicrobial intake, immune status, transit time, have the ability to alter the microbiota composition of the gut. However, the availability of substrate is also a major determinant of composition. Here, the consumer can exert some control, through dietary considerations. Generally, protein and lipid metabolism by the gut flora have a negative impact upon host health. Both are fermented by components of the gut flora and have the effect of generating toxins, including carcinogens. On the contrary, dietary carbohydrates form the principal fermentable substrate for the gut flora and their metabolism produces organic acids which have a harmless, or beneficial, effect. Main carbohydrates involved in gut microbiology are resistant starches, dietary fibres and oligosaccharides. The former two ingredients have been seen as useful because of their ability to cause faecal bulking as a response to increased microbial metabolism. They are therefore advocated to improve digestion and transit time, and have received attention in motility disorders as well as colorectal cancer and diverticulitis.

However, the fermentation of oligosaccharides by gut bacteria is currently a topical area of nutritional sciences that has perceived health bonuses. Certain oligosaccharides have the ability to resist digestion or absorption in the upper gut and are selectively metabolised by the gut flora. If this selection is towards indigenous bifidobacteria and/or lactobacilli then this stimulates their numbers and has an output similar to what is attempted to probiotics. This concept is known as the prebiotic effects. Prebiotics were first defined in 1995 as ‘non digestible food ingredients that are selectively metabolised by colonic bacteria that have the capacity to improve health.’ As such, their use is directed towards favouring beneficial changes in the gut microbial milieu. They are distinct from most dietary fibres like pectin, celluloses, xylan, which are not selectively metabolised in the gut.

Several prebiotics exist and have been confirmed for their (usually bifidogenic) effects in different laboratories. In Europe, prebiotics like fructooligosaccharides (FOS), inulin and galactooligosaccharides (GOS) are increasingly being added to

appropriate food vehicles. Lactulose is also a reported prebiotic in Europe. In Japan, a much wider list exists which includes isomaltooligosaccharides, soyaoligosaccharides, gentiooligosaccharides, glucooligosaccharides, lactosucrose, polydextrose, xylooligosaccharides. These emerging prebiotics are gradually finding their way into the worldwide market. For an efficient prebiotic effect a dose of at least 5g/d seems necessary with studies reporting intakes of up to 30g/d with no adverse affects (too high a prebiotic dose may compromise the selectivity of effect with the consequence of gas generation, which is not a trait associated with bifidobacteria or lactobacilli). Figure 3 shows data from one of our human studies where FOS containing biscuits were ingested and stimulated bifidobacteria to a similar extent which occurs in the breast fed infant. The advantage of prebiotics over probiotics is that concerns regarding product integrity, viability or stability are not issues, hence they can be added to many food vehicles. These include dairy products, beverages and health drinks, spreads, infant formulae and weaning foods, cereals, bakery products, confectionery chocolates, chewing gum, savoury product, soups, sauces and dressings, processed meat products, dried instant foods, canned foods, animal feeds, petfoods and sport nutritional supplements. New product developments are occurring at a rapid pace. They can also be used as powdered or syrup supplements. Such prebiotic containing foods can induce dramatic changes in the gut flora composition.

What are the health consequences? As prebiotics are a newer concept than probiotics, their health values have not been as extensively researched. However, the beneficial natures of both approaches are undoubtedly the same. Active research is ongoing and has been reported for prebiotics in the area of bowel cancer, IBD, IBS, protection against pathogenic agents, coronary heart disease, necrotising enterocolitis, improved mineral bioavailability, autism, vaginal thrush and obesity. As for the probiotics, the trials should be mechanistically driven, well controlled and using the best methodologies available. For the latter, this would involve a molecular approach towards determining gut flora changes in response to diet. This is because the community is far too heavily colonised and complex to rely upon traditional plating procedures. Fluorescent *in situ* hydridisation (Fig. 4), 16S rRNA profiling, T/DGGE, direct community analysis, ribotyping, pulsed field gel electrophoresis, expression arrays, detection genes that affect function, microchips, proteomics, metabolomics and transcription studies are all currently being applied to gut microbiology.

One especially important avenue for prebiotics may lie in food safety issues. Considerable effort and resource is expended at cleaning up the food chain from 'farm to fork' or 'plough to plate.' However, food (and water) borne pathogens have their destructive effects after the fork or plate, i.e. in the gut. It is feasible that a gut flora dominated by bifidobacteria or lactobacilli has the ability to better withstand the effects of transient (and indigenous) pathogens. This is because of several mechanism including acid formation (acetate, lactate), excretion of antimicrobial agents, improved immune status, competition for nutrients and colonisation sites. In this regard, our studies with Bo Llonderdahl's group in Davis, CA showed that primates fed a challenge of enteropathogenic *E. coli* could better withstand the diarrhoea inducing capacity of the pathogen when they were fed prebiotics. In some cases, the protection was as robust as that offered by breast milk. This *in vivo* model system is as close as is feasible to humans and is a good indicator that straightforward additions to the diet can reduce the effects of specific pathogens. In the future, this kind of approach may be extrapolated into other bacterial and viral causes of disorder, including some of the conditions described earlier.

Prebiotics tend to well fermented by bifidobacteria because of their enzyme profile and a preference for oligosaccharide sized substrates. The bifidobacteria are especially adept at FOS utilisation because of a cell-associated β-fructanfuranosidase activity. Changes in response to prebiotics have been mainly detected in the luminal contents of humans. However, a profuse microbiota also exists at the host-mucosal surface. It is easy to envisage that these relatively under researched components have a large impact on gastrointestinal health. In particular, the recent phenomenon of microbe to host 'cross talk' has been elucidated whereby the commensal bacterium *Bacteroides thetaiotaomicron* could elicit the production of fucosylated glycans (Fucα1,2Galβ-glycans) from the host via a molecular sensor. Hence, the microbe was able to engineer its own metabolic niche within the ecosystem. It is not yet known, but the likelihood is that if similar molecular messages can be sent by probiotics and pathogens then they are likely to be different in nature and effect. In any case, the attachment of pathogens to the gut wall would allow the expression of toxins and/or invasion into the colonocyte. In this context, prebiotics may be manufactured to act as

anti-infective agents. Many oligosaccharides are known to act as receptors for gastrointestinal pathogens and their toxins and the idea of using such materials as molecular decoys is gaining currency. To date, however, most attempts to use oligosaccharides to prevent adhesion of pathogens have focussed on a therapeutic approach and the effect on adhesion of the indigenous probiotics has not been extensively investigated. We are interested in examining the affect on microbial adhesion and the prebiotic activity of a range of novel oligosaccharides to optimise the prophylactic management of gut pathogens.

Conclusions

Virtually everyone experiences a gastrointestinal complaint at some time of their lives which can be mediated by microorganisms e.g. gastroenteritis or more chronic ailments listed above. Prebiotics and probiotics have the capacity to help redress this. Even a sceptic would admit that the approaches are relatively harmless (which cannot be said for many gut pharmaceutical approaches including antibiotics) but hold promise. Moreover, even ignoring the beneficial aspects of a lactic acid flora, then displacement of a pathogenic flora with one that is more anodyne in nature should be supported.

Some age groups are more prone to intervention that others, as the magnitude of a prebiotic effect is related to starting levels of the target flora populations (a low number of bifidobacteria respond more readily to prebiotic intake than those which are high). Examples include the elderly, who are especially prone to gastrointestinal infection, weaning children, formula fed infants, frequent travellers, persons ingesting antibiotics and those prone to gastrointestinal complaints like IBS. However, the option is opening up for everyone. In particular, new approaches are required in the developing world where infectious agents are especially troublesome and medical intervention is prohibitively costly.

In terms of how to feed your bacteria with dinner, then a ‘balanced’ high fruit and vegetable intake is key. Many foods like onions, garlic, asparagus, chicory, milk, artichoke, leeks, bananas naturally contain prebiotics. The ingestion of a supplemented food should not be viewed as a replacement for a ‘healthy’ diet but rather an adjunct. It is also important that as the advantages (or otherwise) of prebiotic

use becomes more apparent and recognised, then they should not be overpriced in the market.

Figure 1. Diagrammatic representation of bacteria and physicochemical interactions in different areas of the human gut.

Figure 2. Typical food sources for varying genera of colonic bacteria.

Figure 3. The effects of prebiotic containing biscuits on the predominant gut flora components of 31 healthy adults. Study was of a crossover nature, doubly blind, placebo controlled. Microbiology was carried out using fluorescent in situ hybridisation (the photograph shows this). Courtesy of Dr KM Tuohy.

Figure 4. Fluorescent image of gut bacteria.

Further Reading

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2. www.vtt.fi/virtual/proeuhealth - a collection of European Union funded research projects on probiotics and prebiotics
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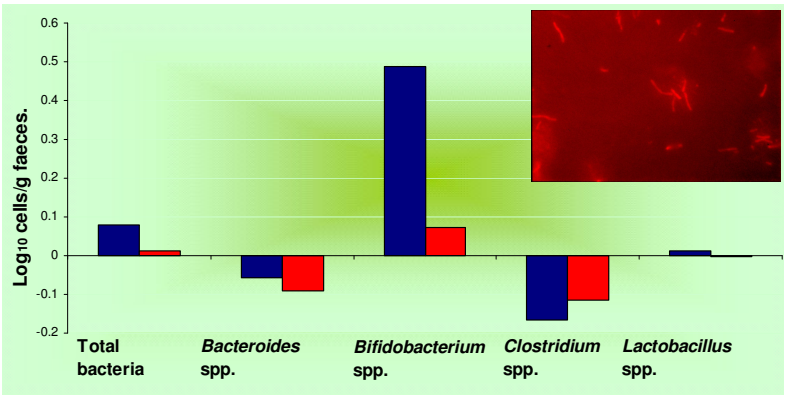

Active biscuits ( ) Placebo biscuits ( )