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Prebiotics, probiotics and human gut microbiology

Laura J. Fooks[a,*], Roy Fuller[b], Glenn R. Gibson[a]

[a]*Department of Food Science and Technology, The University of Reading, Whiteknights, Reading, UK*
[b]*Russet House, Ryeish Green, Reading, UK*

Abstract

Because of its resident microbiota, the human colon is one of the body's most metabolically active organs. Gut bacteria predominantly ferment undigested food materials. The nature of the fermentation may have different health consequences. For example, the end products of carbohydrate metabolism are benign, whilst proteolytic metabolites may be toxic. The use of diet to fortify certain gut flora components is a popular current aspect of functional food sciences. In this context probiotics, prebiotics and synbiotics all have a significant role. Probiotics are live microbial additions to the diet; prebiotics are foodstuffs that have a selective metabolism in the hindgut, whilst synbiotics are combinations of the two approaches. It has been demonstrated that each of these dietary intervention routes can have an effect on the gut flora 'balance'. Whilst the real health advantages remain elusive, the use of gut microflora management has a number of potentially very important effects with resistance to pathogens, effects on gut tumours and reduction in blood lipids holding much promise. The advent of molecular tools into gut microbiology, now offer the means to more fully explore the gut biodiversity as well as reliably track changes in response to diet. The near future will determine whether the full potential of probiotics, prebiotics and synbiotics can be realised. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Human gastrointestinal bacteriology

Functionally, the human colon undertakes a number of important physiological activities. For example, enterocytes actively transport sodium and chloride, with absorption occurring via a parallel mechanism of sodium–hydrogen and chloride–bicarbonate exchanges. Potassium enters the lumen via enterocytes, utilising an active secretory pathway. The mucosa is rich in carbonic anhydrase and in endocrine cells which produce hormones and neurotransmitters. As such, physiologically and endocrinologically, the human colon has major importance. However, another extremely significant metabolic trait is mediated by gut bacteria.

In the human gastrointestinal tract, there exists variability in bacterial numbers and populations between the stomach, small intestine and colon. The total bacterial count in gastric contents is usually below 10^3 per g, with numbers in the small intestine ranging from about 10^4 per ml of contents to about 10^6–10^7 at the terminal ileum (Gorbach, Nahas & Lerner, 1967).

In comparison to other regions of the gastrointestinal tract, the human large intestine is a complex, heavily populated and diverse microbial ecosystem. Bacterial numbers in the human large intestine are in the region of 10^{11}–10^{12} for every gram of gut contents (Cummings & Macfarlane, 1991).

The colonic microflora is capable of responding to anatomical and physicochemical variations that are present. The right or proximal colon is characterised by a high substrate availability (due to dietary input), a pH of around 5.5–6.0 (from acids produced during microbial fermentation) and a more rapid transit than the distal region. The left, or distal, area of the colon has a lower concentration of available substrate, the pH is approximately 6.5–7.0 and bacteria grow more slowly. The proximal region tends to be a more saccharolytic environment than the distal gut, the latter having higher bacterial proteolysis. Several hundred different species of bacteria are thought to be present in the large intestine. Gram negative rods belonging to the *Bacteroides fragilis*

* Corresponding author. Tel.: +44-11-89357033; fax: +44-11-89267917.
E-mail address: laura.fooks@bbsrc.ac.uk (L.J. Fooks)

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group are the numerically predominant culturable bacteria in the colon. The other main groups consist of different (Gram positive) rods and cocci, such as bifidobacteria, clostridia, peptococci, streptococci, eubacteria, lactobacilli, peptostreptococci, ruminococci, enterococci, coliforms, methanogens, dissimilatory sulphate-reducing bacteria and acetogens. The flora includes saccharolytic organisms, proteolytic species and bacteria which can metabolise gases. Despite the huge diversity of bacteria thought to be present in the large gut (ca. 400 described species), it is certain that the vast majority has not been hitherto identified. Future studies that exploit a molecular approach to the fermentation will help unravel this 'hidden' diversity. A number of different factors are able to affect the composition of the colonic microbiota and some examples are given in Table 1.

Predominant growth substrates for gut bacteria are of dietary origin and consist of foodstuffs that have not been absorbed in the upper gastrointestinal tract. These include resistant starches, dietary fibre, sugars, oligosaccharides, proteins, peptides and amino acids. There is also a quantitatively lower contribution from endogenous sources such as mucins.

Principal end products of bacterial fermentation in the colon are short chain fatty acids (SCFA), i.e. acetate, propionate and butyrate. Other fermentation products include ethanol, lactate, succinate, formate, valerate and caproate. Branched chain fatty acids such as isobutyrate, 2-methyl-butyrate and isovalerate may also be formed from the fermentation of amino acids. Most of the SCFA formed by intestinal bacteria are absorbed, and systematically metabolised, thereby contributing towards host energy gain (Cummings, 1995).

Unlike carbohydrate metabolism, where the end products are benign and may even be of some benefit to the host, those from proteolysis are toxic. These include ammonia, phenols, indoles and amines (Macfarlane & Macfarlane, 1995).

Table 1
Examples of factors which may affect the composition of the human gut microflora

Type of feeding
Amount, chemical composition and availability of growth substrates
Availability of colonisation sites
Immunological interactions
Individual fermentation strategies by the bacteria
Intestinal transit time
Gut pH
Redox potential
Availability of inorganic electron acceptors
Production of bacterial metabolites
Presence of antimicrobial compounds
Xenobiotic compounds
Age of the host
Peristalsis

2. Microflora balance

The bacterial populations which inhabit the intestines have adapted such that the numbers of each genera are approximately consistent, with each having their own growth niche. In order for the intestine to function optimally the 'balance' of the bacteria must be maintained, and this appears to be increasingly difficult as lifestyles change. Various factors may shift the balance of the gut microflora away from potentially beneficial or health promoting bacteria such as lactobacilli and bifidobacteria, and towards a predominance of potentially harmful or pathogenic microorganisms, like clostridia, sulphate-reducers and certain *Bacteroides* species. Predominance of these latter populations may pre-dispose to a number of clinical disorders, including cancer, inflammatory disease, ulcerative colitis, whilst making the host more susceptible to infections by transient enteropathogens like *Salmonella*, *Campylobacter*, *Escherichia coli* and *Listeria*. It is of considerable benefit to the host therefore, to maintain a good community structure through increased predominance of bacteria such as lactobacilli and bifidobacteria. A recent review by Bengmark (1998) has highlighted the importance of so called 'microbial interference treatment' as means of infection control:

- A recognition that antibiotic-therapy has not been successful to the extent that may have been expected. Although it has no doubt solved some medical problems, it has also created some new ones.
- An increased awareness of the fact that antibiotic treatment deranges the protective flora, and thereby predisposes to later infections.
- An increase in antibiotic resistant microbial strains, as a result of widespread overprescription and general misuse.
- A fear that industry will no longer be able to develop effective antibiotics at a sufficient rate to compete with the development of microbial resistance to old antibiotics.
- Widespread public interest in ecological methods.

Each of the points raised here give justification to the use of probiotics, to help protect from various intestinal diseases and disorders. What remains to be established is the extent to which these probiotic organisms can be beneficial, to determine how any benefits may be manifested and to recognise any limitations.

3. Definitions

The word probiotic is derived from two Greek words meaning 'for life'. Early attempts to use the term to mean a microbial substance which stimulates the growth of another microorganism (Lilley & Stillwell, 1965) or tissue

extracts which improved microbial growth (Sperti, 1971) did not gain general acceptance. Parker (1974) first used the word probiotic in the context of animal feed supplementation and defined it as:

Organisms and substances which contribute to intestinal microbial balance.

Fuller (1989) redefined probiotics by removing the reference to 'substances' which could include antibiotics and microbial stimulants. His revised definition is:

A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.

This modified version stresses the need for the supplement to be composed of viable microorganisms and is the most widely accepted probiotic definition.

A probiotic effect is, therefore, mediated through the gut microflora by ingestion of viable microorganisms. Such a definition entails preparations specifically designed for probiotic use as well as traditional yoghurts and other fermented foods. In 1998, the European probiotic yoghurt market alone was estimated to be worth a value in the region of £520 million (Shortt, 1998), with the UK market reported as being the fastest growing. Probiotic products currently on the market are presented in the form of powders, tablets or capsules, liquid suspensions and sprays. Most preparations destined for human consumption are in fermented milks or given as powders or tablets. They can contain one or several species of bacteria or fungi.

4. Development of the probiotic concept

Although the word probiotic by definition was not established until 1965, the concept was worked upon by Metchnikoff at the beginning of the century. Metchnikoff had for a long time believed that the complex microbial population in the colon was having an adverse effect on the host through so-called 'autointoxication'. In 'The Prolongation of Life' he reported that Bulgarian peasants, who consumed large quantities of fermented milk had longevity. As such, Metchnikoff began to modify the colonic microflora through ingestion of soured milks. He used a Gram-positive rod which he called the Bulgarian bacillus and later *Bacillus bulgaricus*. It is probable that this organism later became known as *Lactobacillus bulgaricus* and is now called *L. delbrueckii* subsp. *bulgaricus* which together with *Streptococcus thermophilus* is responsible for the traditional fermentation of milk into yoghurt.

A number of scientific progressions then ensued to reach the current situation whereby many probiotic strains exist and are in widespread use. Table 2 shows lactic acid bacteria which are currently being used in probiotic preparations either singly or in combination.

Table 2
Examples of lactic acid bacteria used as probiotics for human consumption

Lactobacilli	Bifidobacteria	Streptococci	Enterococci
L. delbrueckii subsp *bulgaricus*	*Bif. bifidum*	*S. thermophilus*	*Ent. faecalis*
L. acidophilus	*Bif. longum*		*Ent. faecium*
L. rhamnosus	*Bif. breve*		
L. reuteri	*Bif. infantis*		
L. casei			

Although adhesion of bacteria to the gut epithelium is an established colonisation factor, it does not ensure that an organism will permanently colonise. However, some strains are more persistent than others. McCartney, Wenzhi and Tannock (1996) have located in the human intestine, indigenous strains of lactobacilli and bifidobacteria which were detectable over a period of 12 months. Strains such as these may prove to be very effective probiotics, generating responses over a long period. Even if, in the short term, a strain is being replaced it may be advantageous to use an adhering strain so that the period of residence in the gut is maximised. Under such conditions, continuous administration ensures the presence in the gut of large numbers of metabolising cells and epithelial adhesion would be less important.

5. Probiotic effects

A number of benefits in the ingestion of probiotics have been reported. These include the following.

5.1. Lactose malabsorption

Lactose malabsorption results from insufficient activity of lactase in the human gut and causes abdominal distension, excessive flatulence and/or diarrhoea. Over half the world's population is unable to utilise lactose effectively. It has been established that lactose administered in yoghurt can be utilised more efficiently than the same amount given in untreated milk (Savaiano, Abdelhak AbouElanouar, Smith & Levitt, 1984). Moreover, probiotic strains can produce β-galactosidase which improves tolerance to lactose.

5.2. Intestinal infections

For a review on the use of probiotics to treat intestinal infections see Gibson, Saavedra, Macfarlane & Macfarlane (1997). One of the most popular areas is that of antibiotic associated diarrhoea (AAD). The efficacy of *Saccharomyces boulardii* given in combination with antibiotics as compared to those given antibiotics alone has

been demonstrated (Adams, Barret, Barret-Bellet, Benedetti, Calendini & Daschen, 1977). The effect of *S. boulardii* treatment in reducing incidence of AAD has also been confirmed (Surawicz, Elmer, Speelman, McFarland, Chinn & van Belle, 1989; McFarland et al., 1995).

Other trials have looked at the treatment of *Clostridium difficile* related infections. For example, recurrence of this infection was significantly reduced in a group given *S. boulardii* with antibiotic treatment, as compared to the antibiotics alone (McFarland, Surawicz, Greenberg, Fekerty, Elmer & Moyer, 1994).

Overgrowth of candida in the gut is also a frequent consequence of antibiotic use. Studies in hamsters have shown that the gut microflora is involved in suppression of *Candida albicans* (Kennedy & Volz, 1985). In gnotobiotic mice, *S. boulardii* protected against colonisation of the gut by this organism (Ducluzeau & Bensaada, 1982). A human trial showed that milk containing *L. acidophilus* and a *Bifidobacterium* was effective in reducing *Candida* occurrence in faeces (Tomoda, Nakano & Kageyama, 1983).

There is also evidence that *Lactobacillus* GG can influence the cause of diarrhoea in children (Isolauri, Juntunen, Rautanen, Sillanaukee & Koivula, 1991).

For probiotics containing bifidobacteria, there have been positive data against AAD (Colombel, Corot, Neut & Romond, 1987; Orrhage, Brismar & Nord, 1994), *Cl. difficile* (Corthier, Dubos & Raibaud, 1985) and childhood forms of diarrhoea (Hotta, Sato & Iwata, 1987; Saavedra, Bauman, Oung, Perman & Yolken, 1994). One particular *B. bifidum* strain has been examined with regard to its efficacy in baby and toddler milk preparations (Haschke et al., 1998).

The mechanism by which protection is offered by these probiotics has not yet been fully established. However, one or more of the following are possible:

- competition for nutrients,
- secretion of antimicrobial substances,
- reduction of gut pH through SCFA formation,
- blocking of adhesion sites,
- attenuation of virulence,
- blocking of toxin receptor sites,
- immune stimulation,
- suppression of toxin production.

5.3. Suppression of cancer

Epidemiological studies have suggested that an increase in the consumption of saturated fats has contributed towards the increased incidence of colon cancer in the Western world. However, no definitively successful clinical trials using probiotics in cancer therapy have been carried out. Animal models have shown that dietary intake of lyophilised cultures of *Bifidobacterium longum* significantly suppressed the development of azoxymethane-induced aberrant cryptic foci (ACF) formation in the colon (Kulkarni & Reddy, 1994). More recently, the same group elucidated the ability of the same strain to inhibit IQ-induced incidence of colon tumours in rats (Reddy, 1998). This was confirmed by another group, which used animal models to determine that a combination of *B. longum* and the prebiotic inulin was effective in generating beneficial changes related to tumour risk (Rowland, Rumney, Coutts & Lievense, 1998). Another team, again using animal models, found that culture supernatants of *L. acidophilus* and *B. adolescentis* suppressed ileal ulcer formation (Kinouchi et al., 1998). However, the problems of transposing observations from rodents, or in vitro cell lines, to humans should be borne in mind.

Bacterial enzymes which convert precarcinogens to active carcinogens are produced in the gut, but their involvement in the pathogenesis of cancer is unclear. However, *L. acidophilus* when fed to healthy volunteers has been shown to significantly decrease β-glucuronidase, nitroreductase and azoreductase activities (Goldin & Gorbach, 1984).

Although mechanisms for the anti-tumour actions of probiotics have not yet been confirmed, some proposals are as follows (McIntosh, 1996):

- suppression of the carcinogen/procarcinogen by binding, blocking, or removal,
- suppression of bacteria with enzyme activities that may convert procarcinogens to carcinogens,
- reducing the intestinal pH, thereby altering microflora activity and bile solubility,
- altering colonic transit time to remove faecal mutagens more effectively,
- stimulation of the immune system.

5.4. Coronary heart disease

Studies to demonstrate that probiotic supplementation can affect plasma cholesterol concentrations and consequently the incidence of coronary heart disease, have given variable data and no firm conclusions can be drawn. However, this area of research has attracted favour as the results are measurable, i.e. any change in plasma total or LDL cholesterol levels can be determined in response to dietary intervention. Schaafsma, Meuling, van Dokkum and Bouley (1998) found that daily feeding of 125 ml test probiotic milk significantly lowered serum LDL cholesterol levels and total serum cholesterol. Similar results have been found by Agerbaek, Gerdes and Richelsen (1995) and Gilliland, Nelson and Maxwell (1985). Potentially, a probiotic may:

- interfere with cholesterol absorption from the gut,
- directly assimilate cholesterol,

- produce metabolites that affect the systemic levels of blood lipids.

5.5. Digestive aid

It is thought that probiotics help the digestion of food materials. This would be directly related to their viability and ability to colonise effectively.

5.6. Nutritional effects

The nutritional content of a fermented milk is little different from that of the raw milk from which it is made (Anon, 1997). The fermentation process may also increase protein availability by the proteolytic action of the starter bacteria.

5.7. Immune stimulation

One of the most interesting aspects of probiotic supplementation is directed towards the immune response. In a human trial, 24 subjects were fed 450 g of yoghurt per day for 4 months, and showed significant increase in the production of γ-interferon (Halpern, Vruwink, Van de Water, Keen & Gershwin, 1991). In animal models, probiotics have been shown to stimulate the production of antibodies (local and systemic), enhance the activity of macrophages, increase γ-interferon levels and increase the concentration of natural killer cells. Obviously, the non-pathogenic nature of the probiotic is crucial.

6. Mechanisms of probiotic activity

The exact manner in which probiotics may achieve their effect(s) is still uncertain. However, a number of mechanisms may be speculated upon:

6.1. Biochemical effects

One mechanism by which organisms may inhibit one another is via the production of bacteriocins (Meghrous, Euloge, Junelles, Ballongue & Petitdemange, 1990). Gibson and Wang (1994) suggested that some strains of bifidobacteria could inhibit a variety of pathogenic bacteria, through a non-pH related effect. However, *B. bifidum* was found to excrete a bacteriocin which affected *Listeria*, *Enterococcus*, *Bacillus*, *Lactobacillus*, *Leuconstoc* and *Pediococcus* (Anand, Srinivasan & Rao, 1984). Other bacterial species known to produce bacteriocins, include lactobacilli and lactococci (Talarico & Dobrogosz, 1989).

Short chain fatty acids (SCFA) are also produced in varying quantities as metabolic end products by probiotic bacteria, and it is thought that these may induce an antagonistic effect against other organisms. A lowering of the gut pH may act directly to inhibit the growth of harmful and pathogenic organisms.

6.2. Competition for nutrients

Certain bacterial species have exact nutritional requirements and it is likely that one population utilises nutrients at the expense of other species. This competitive interaction may be fortified through probiotic use.

6.3. Immune effects

The potential of the immune response to control the growth of micro-organisms in the gut is an important consideration. Further work may establish how stimulation of the systemic components of the immune system, through non-pathogenic means, can help regulate the gut microflora.

6.4. Colonisation

One possible mechanism for the action of probiotics is their ability to adhere to the intestinal mucosa. As such, they can resist peristalsis and occupy a niche at the expense of harmful organisms.

7. Prebiotics

As the viability of live bacteria in food products and during transit through the gastrointestinal tract may be variable, the prebiotic concept has been developed. Here, a selective growth of indigenous gut bacteria through the diet is required. *A prebiotic is 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 in the colon, that can improve host health* (Gibson & Roberfroid, 1995). Thus, the prebiotic approach advocates administration of non-viable entities and aims to overcome survival problems in the upper gastrointestinal tract. Certain oligosaccharides which cannot be digested, except through bacterial activity, are prebiotics. Those that contain fructose (e.g. inulin) are able to alter the composition of the human gut flora towards a predomination of bifidobacteria.

Criteria which allow the classification of a food ingredient as a prebiotic, include:

(1) It must be neither hydrolysed, nor absorbed in the upper part of the gastro-intestinal tract.

(2) Selective fermentation by potentially beneficial bacteria in the colon.

(3) Alteration in the composition of the colonic microbiota towards a healthier composition.

(4) Preferably, induce effects which are beneficial to the host health.

Any foodstuff that reaches the colon, e.g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids, is a candidate prebiotic. Certain non-digestible carbohydrates seem authentic prebiotics. Fructo-oligosaccharides (FOS) are β-d-fructans with various degrees of polymerisation. A number of other non-digestible oligosaccharides have now been developed, for which there is some evidence of their prebiotic effect. These include gluco-oligosaccharides, galacto-oligosaccharides, transgalacto-oligosaccharides, isomalto-oligosaccharides, xylo-oligosaccharides, and soybean-oligosaccharides (Gibson, Rastall & Roberfroid, 1999; Hayakawa, Mizutani, Wada, Masai, Yoshihara & Mitsuoka, 1990; Ito et al., 1990; Ito, Kimura, Deguchi, Miyamori-Watabe, Yajima & Kan, 1993; Imaizumi, Nakatsu, Sato, Sedamawati & Sugano, 1991; Kohmoto, Kukui, Takaku, Machida, Arai & Mitsuoka, 1988; Saito, Takano & Rowland, 1992).

Of all the possible prebiotics, the inulin type fructans have been the most thoroughly investigated. The fermentability of various dietary components has been studied in vitro using mixed faecal culture, with the predominant culturable bacterial groups, including bacteroides, clostridia, lactobacilli and bifidobacteria being enumerated (Wang & Gibson, 1993). Bifidobacteria selectively fermented the fructans, in preference to other carbohydrate sources such as starch, fructose, pectin and polydextrose. This was subsequently confirmed in a volunteer trial, which examined the bifidogenic effect of fructo-oligosaccharides (Gibson, Beatty, Wang & Cummings, 1995). These data have been confirmed in other human studies (Buddington, Williams, Chen & Witherly, 1996; Kleesen, Sykura, Zunft & Blaut, 1997).

Gluco-oligosaccharides (GOS) have as yet not been extensively investigated. Although they are thought to be bifidogenic, one study fed gnotobiotic rats a diet of 40 g/d of GOS and found little effect on the bacterial groups. However, they did modify certain glycolytic activities (Djouzi & Andrieux, 1997).

Transgalacto-oligosaccharides (TOS) are manufactured from lactose by transglycosylation reactions and consist of galactosyl derivatives of lactose with β1-3 and β1-6 linkages. Bifidobacterial numbers were significantly increased in the faeces of rats fed TOS (Djouzi & Andrieux, 1997), confirming data from earlier experiments (Tanaka et al., 1983; Mitsuoka, 1990). One study used transgalactosylated disaccharides in a human volunteer trial to determine their effects on the faecal flora. This showed that the prebiotic increased bifidobacteria and *Lactobacillus* numbers, whilst decreasing *Bacteroides* sp. and *Candida* sp. (Ito et al., 1993). The bifidogenic nature of TOS has been related to a linkage specificity of the *Bifidobacterium* β-galactosidase, which cleaves β1-3 and β1-6 linkages, instead of β1-4 linkages (Dumortier, Brassart & Bouquelet, 1994).

Iso-maltooligosaccharides (IMO) have been shown to be fermented by bifidobacteria and *Bacteroides fragilis*, but not by *E. coli* and other bacterial populations (Kohmoto et al., 1988). Our own (unpublished) results with an in vitro model of the human gut have indicated that IMO are very efficient prebiotics in that they stimulate a lactic microflora as well as allow elevated production of butyrate, which is thought to be a desirable metabolite in the gut. Other studies have looked at soybean-oligosaccharides, raffinose and stachyose (Hayakawa et al., 1990). All bifidobacteria species tested fermented this carbohydrate, with the exception of *B. bifidum*, whilst *L. salivarius*, *Bact. fragilis* and *Mitsuokella multiacida* metabolised the substrate to a lesser degree.

These results suggest that all of these oligosaccharides have the potential for being used for human ingestion, to enhance numbers of bifidobacteria. Because evidence exists for their purported positive effects, prebiotics can justifiably be used as a dietary supplement to alter the gut flora composition.

The inhibition of growth of some human enteropathogens such as salmonellae has been reported in the presence of FOS (Oyarzabal & Conner, 1995), whilst others report bifidobacterial antagonistic activity against Gram-negative species, like *Salmonella*, *Campylobacter* and *E. coli* (Gibson & Wang, 1994). Since salmonellae and campylobacters are very common aetiological agents in gastroenteritis and diarrhoeal disease, there is clearly a market potential for the prophylactic or direct treatment. However, these organisms cause fewer fatalities than *E. coli*. The 1999 outbreak of *E. coli* 0157 in North Cumbria with 27 people hospitalised comes only three years after the poignant Lanarkshire outbreak in which 21 pensioners died. These and other smaller, isolated, cases highlight the importance of continuing any investigations which could offer some protection, in terms of administering prebiotics to enhance the numbers of beneficial micro-organisms. Laboratory studies (Fooks et al., unpublished data) have shown that bifidobacteria are very powerful inhibitors of *E. coli* 0157.

8. Synbiotics

A further possibility in microflora management procedures is the use of synbiotics, where probiotics and prebiotics can be used in combination. The live microbial additions may be used in conjunction with a specific substrate for growth, for example FOS with a *Bifidobacterium* strain. The end result should be improved survival of the probiotic, which has a readily available (and specific) substrate for its fermentation, as well as the individual advantages that each should offer.

The approach could have particular application in babies and the elderly. An accumulation of literature has reported the benefits of breast-feeding infants, as

compared to bottle-feeding in terms of the predominance of different bacterial groups. Administration of bifidobacteria with a bifidogenic prebiotic, could improve numbers in the intestine.

In elderly individuals, above the age of about 55 yr, faecal bifidobacterial counts are thought to show a marked decrease in comparison to those of younger persons (Mitsuoka, 1990; Kleesen et al., 1997). This may be relevant in the susceptibility of these individuals to pathogenic infection. Victims in the *E. coli* outbreaks mentioned above were predominantly children, and adults over the age of 55. This possibly highlights colonisation of the intestine as a mechanism of the action of these bacteria, since, as bifidobacterial numbers decrease, this may leave more opportunity for enteric pathogens to colonise. As nearly half of the UK population is comprised of children and adults over the age of 60, there is a substantial target population.

9. Application of molecular techniques in intestinal microbiology

It is well recognised that the large gut hosts a complex and diverse ecosystem, and whilst much is already known about this environment, we have yet to determine the exact complexity and magnitude of the bacterial populations which reside therein. Until recently most studies used culturing techniques, based on reportedly selective agars, to plate out samples and then bacterial colonies are enumerated and phenotypically characterised. This technique relies on the culturability of the species being examined, and as such, any microorganisms unable to grow on the agar selected, or under the in vitro conditions applied, would not be detected. This therefore leads to an underestimation in the bacterial population in relation to the total present. In addition, the technique assumes selectivity of the agar used for enumeration, which is virtually impossible, and cannot differentiate probiotic organisms, such as lactobacilli and bifidobacteria, from commensal counterparts. There is a need therefore to develop techniques which could more accurately present information about the gut microflora.

16S rRNA is a molecular chronometer which is revolutionising bacterial diversity and constantly discovering new taxa. Moreover, 16S rRNA sequencing data provides essential information for developing molecular based tests for identifying specific bacterial populations, without the need to directly cultivate. By exploiting different regions of conservation within the 16S rRNA it is possible to identify specific characteristics in different taxa that can act as targets for gene probes.

Oligonucleotide probes have been developed at the genera- and species-specific levels, which hybridise to organisms with that complementary sequence. The procedure for developing a genus-specific probe involves: (i) comparison of sequences by alignment; (ii) identification of a target sequence which is unique for the genus; (iii) synthesis and labelling of complementary nucleic acid probes; (iv) experimental evaluation of the probe (Welling, Elfferich, Raangs, Wildeboer-Veloo, Jansen & Degener, 1997).

10. Conclusion

Whilst the market for probiotic containing products has enjoyed a substantial increase in popularity recently, the basis for their action has not yet been firmly established. Evidence from in vitro studies and human volunteer trials suggest beneficial effects, in a number of clinical applications, but considerable progress has yet to be made, in terms of both effects on host health and mechanisms of action.

Prebiotics have also been investigated since their effects on the indigenous bacterial populations can be more easily examined. As more reliable and informative procedures are developed, the formation of synbiotics which promote host health can be applied, in both maintaining the balance of the gut flora in healthy individuals, and restoring the equilibrium in individuals whose gastrointestinal microbiota has been altered as a result of illness and/or disease, age, or diet.

11. For Further Reading

The following reference is also of interest to the reader: Sghir et al., 1998.

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