

Review Article

Intestinal microbiota, diet and health

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(Submitted 11 December 2012 – Final revision received 3 July 2013 – Accepted 3 July 2013 – First published online 12 August 2013)

Abstract

The human intestine is colonised by 10^{13} to 10^{14} micro-organisms, the vast majority of which belong to the phyla Firmicutes and Bacteroidetes. Although highly stable over time, the composition and activities of the microbiota may be influenced by a number of factors including age, diet and antibiotic treatment. Although perturbations in the composition or functions of the microbiota are linked to inflammatory and metabolic disorders (e.g. inflammatory bowel diseases, irritable bowel syndrome and obesity), it is unclear at this point whether these changes are a symptom of the disease or a contributing factor. A better knowledge of the mechanisms through which changes in microbiota composition (dysbiosis) promote disease states is needed to improve our understanding of the causal relationship between the gut microbiota and disease. While evidence of the preventive and therapeutic effects of probiotic strains on diarrhoeal illness and other intestinal conditions is promising, the exact mechanisms of the beneficial effects are not fully understood. Recent studies have raised the question of whether non-viable probiotic strains can confer health benefits on the host by influencing the immune system. As the potential health effect of these non-viable bacteria depends on whether the mechanism of this effect is dependent on viability, future research needs to consider each probiotic strain on a case-by-case basis. The present review provides a comprehensive, updated overview of the human gut microbiota, the factors influencing its composition and the role of probiotics as a therapeutic modality in the treatment and prevention of diseases and/or restoration of human health.

Key words: Intestinal microbiota: Diet: Health: Probiotics

The human intestinal microbiota plays a key role in numerous metabolic, physiological, nutritional and immunological processes⁽¹⁾, and perturbations in the composition of the microbiota influences human health⁽²⁾. Much of the early information regarding the intestinal microbiota has come from studies that used culture-dependent techniques, which reveal only a minority of species constituting the microbial population^(2,3). However, the advent of culture-independent, DNA-based analyses has generated data that can be mined for information on the composition and functional properties of this hitherto-uncultured microbiota^(2,4,5).

The microbial content of the gastrointestinal tract (GIT) changes along its length, ranging from a narrow diversity and low numbers of microbes in the stomach to a wide diversity and high numbers in the large intestine^(6,7) (Fig. 1). The best-studied region of the gut is the distal colon, and in

adults, faeces-derived populations have been estimated to consist of 10^{13} to 10^{14} micro-organisms, composed of approximately 1100 prevalent species, with at least 160 such species per individual. In its entirety, the microbiota is estimated to contain 150-fold more genes than the human genome⁽⁸⁾. The majority of bacteria belong either to the phylum Firmicutes (including *Clostridium*, *Enterococcus*, *Lactobacillus* and *Ruminococcus*) or to the phylum Bacteroidetes (including *Bacteroides* and *Prevotella* genera), which constitute over 90% of the known phylogenetic categories found in the human intestine^(8–14). Although there is huge inter-individual variability in microbial compositions^(8,9,12,15), recent work has revealed that a core group of more than fifty taxa can be found in nearly half of the human subjects sampled^(8,13). It has also been suggested that the microbiota of most individuals can be categorised into three predominant variants, or 'enterotypes',

Abbreviations: AAD, antibiotic-associated diarrhoea; CD, Crohn's disease; EPS, exopolysaccharide; GF, germ free; GIT, gastrointestinal tract; HC, healthy controls; IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome; LPS, lipopolysaccharide; UC, ulcerative colitis.

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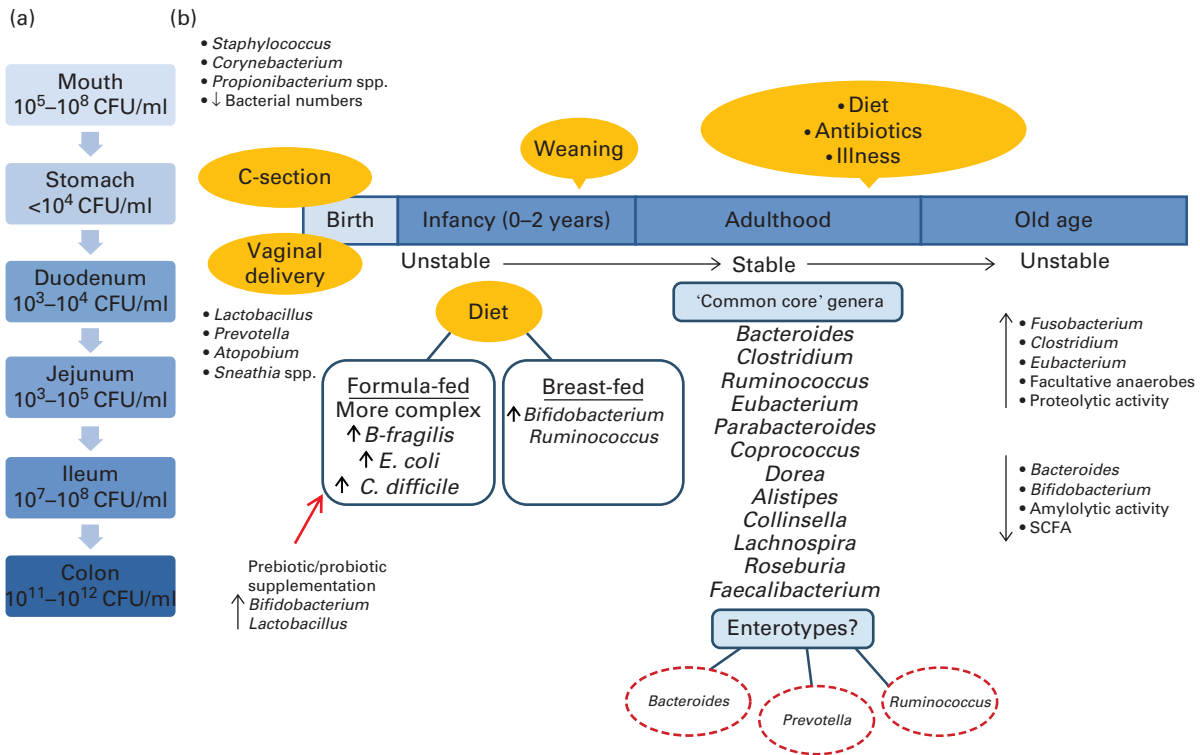


Fig. 1. (a) Variations in microbial numbers across the length of the gastrointestinal tract. (b) Selected features affecting the establishment and maintenance of the microbiota and factors influencing the composition of the microbiota. Micro-organisms are listed where their abundance is related to a particular environmental factor^(6–8,13,16,25,26,45,173). C-section, Caesarean section; CFU, colony-forming units; *B. fragilis*, *Bacteroides fragilis*; *E. coli*, *Escherichia coli*; *C. difficile*, *Clostridium difficile*.

dominated by three different genera: *Bacteroides*; *Prevotella*; *Ruminococcus*, which are independent of age, sex, nationality and BMI⁽¹⁶⁾. This concept was partially supported by Wu *et al.*⁽¹⁷⁾, who identified two enterotypes, distinguished primarily by the levels of *Bacteroides* and *Prevotella*, which were largely driven by diet. More recently, considerable debate has arisen about the notion of enterotypes^(18,19), with a number of studies^(20,21) failing to identify the three distinct categories described by Arumugam *et al.*⁽¹⁶⁾. Researchers are now favouring the idea of a continuum or gradient of species functionality rather than a discontinuous variation with segregated types⁽¹⁹⁾.

Studies have also identified a core microbiome at the gene rather than at the organismal lineage level^(22,23). These studies suggest that, rather than a core group of species, individuals share a core group of microbiome functions and individuals exhibiting particular phenotypes (e.g. obese or non-obese) may display different patterns of gut microbes but share a core group of functions⁽²³⁾. Changes in this core set of genes may account for different states of health and disease. Future research will investigate whether the metagenome predicts a risk for developing particular human diseases to obtain new microbial diagnostic markers that may allow early diagnosis of diseases and development of potentially new therapeutic strategies.

Although much has been discovered in the last decade about the intestinal microbiota, there are biases and limitations to the current knowledge related to study design, sample collection and confounding variables, such as diet. Investigations into the impact of diet on the intestinal

microbiota are challenged by the inability of researchers to carry out large-scale, carefully controlled trials in humans. There is also a need to better clarify the mechanisms through which changes in microbial dysbiosis promote disease states to improve our understanding of the causal relationship between the gut microbiota and disease. Whether a disease-prone microbial composition can be transformed into a healthier composition by probiotic/prebiotic/dietary interventions remains a fundamental unanswered question. Nonetheless, the emergence and growing accessibility of the next-generation sequencing technologies will greatly advance the discovery of the composition and functional capacity of microbial communities in the human gut. In the present review, we discuss recent insights into the impact of age, diet, antibiotic use and disease on the intestinal microbiota. We also highlight the role of probiotics, in particular, the potential role of dead or inactivated microbes as a therapeutic modality in the treatment and prevention of diseases and/or restoration of human health.

Factors influencing the composition of the microbiota

Age

The development of the human microbiota is a dynamic process, with individuals exhibiting differences in terms of microbial diversity and variation at different life stages (Fig. 1)^(2,6,24). The human microbiota is established at birth when the intestine becomes inhabited by a population that

is characterised by instability⁽⁶⁾. Initially, facultative anaerobes such as Enterobacteriaceae, streptococci and staphylococci dominate⁽²⁵⁾. In recent years, more stringent hygienic conditions during delivery combined with shorter hospital stays have reduced bacterial exposure, leading to changes in the initial colonisation pattern, with skin-derived staphylococci being the first colonisers of the infant gut rather than faecal Enterobacteriaceae^(25,26). For infants born vaginally, the first encounter with micro-organisms occurs in the birth canal, where colonisation is initiated by the maternal vaginal and intestinal microbiota as well as the environment⁽²⁷⁾. In contrast, for infants delivered by caesarean section, the environment (e.g. nursing staff and the air) is an extremely important source of colonising bacteria, and these infants have lower intestinal bacterial counts with less diversity in the early weeks of life^(26,28,29).

Other factors influencing the microbiota include gestational age, hospitalisation of the infant, antibiotic use and infant feeding. Breast-fed infants have a microbiota dominated by *Bifidobacterium*^(30–33) and *Ruminococcus*^(26,34), with the rates of colonisation by *Escherichia coli*, *Clostridium difficile*, *Bacteroides fragilis* group bacteria and lactobacilli being significantly lower than those observed in exclusively formula-fed infants^(31,35). The microbiota of formula-fed infants is more complex^(30,36) and comprises a variety of bacterial genera, including enterobacterial genera, *Streptococcus*, *Bacteroides* and *Clostridium*, as well as *Bifidobacterium*⁽³⁴⁾ and *Atopobium*⁽³⁰⁾. It must be noted that some reports have found no differences in the compositions of the microbiota between breast-fed and formula-fed infants and have attributed this to modern formulas more closely mimicking the composition of human breast milk⁽³⁾. The composition of the microbiota changes further with the introduction of solid foods, and a complex, more stable community similar to the adult microbiota becomes established after weaning (at 2–3 years of age)^(33,34,37,38).

Throughout adulthood, the composition of the intestinal microbiota is relatively stable and is only transiently altered by external disturbances⁽³⁹⁾, as will be discussed below. However, this relative stability is reduced in old age⁽⁴⁰⁾. There is considerable variation in the reported microbial compositions of elderly subjects, which appear to be dependent on residence cohort, geographical location and detection methods used^(6,41). The large inter-individual variation in microbial compositions continues into old age⁽⁴²⁾, and the process of ageing coincides with the decreasing diversity of the microbiota⁽⁴³⁾. Researchers are continually striving to elucidate the composition of the intestinal microbiota of the elderly, but as yet no specific ‘common core’ has been identified. However, some of the fundamental changes that occur include a decrease in the total number and species diversity of bifidobacteria and *Bacteroides* as well as a reduction in amylolytic activity and the availability of total SCFA. There is a concurrent increase in the number of facultative anaerobes, fusobacteria, clostridia and eubacteria as well as an increase in proteolytic activity^(44,45). A study by the ELDERMET consortium has found that the microbial population of elderly Irish subjects is dominated

by Bacteroidetes, whereas the microbiota of younger subjects is dominated by Firmicutes⁽⁴²⁾. However, Biagi *et al.*⁽⁴³⁾ did not find significant differences among the Firmicutes: Bacteroidetes ratios of Italian centenarians, elderly and young adults. These conflicting results have been attributed to the country-related variation in the compositions of the gut microbiota⁽²⁴⁾, which has been highlighted several years ago⁽⁴¹⁾ and presumably may be linked to the diet. Furthermore, the composition of the gut microbiota of the elderly may also vary depending on residence location^(20,46), which is a proxy measure for radically different diets (see below).

Diet

Diet is a factor that undoubtedly influences the composition of the intestinal microbiota. Diet provides nutrients for both the host and the bacteria in the GIT. Most of the enzymes needed to break down the structural polysaccharides in plant material are not encoded by mammalian genomes. The intestinal microbiota produces a larger collection of degradative enzymes and exhibits a broader range of metabolic capabilities⁽⁴⁷⁾. It is estimated that 20–60 g of dietary carbohydrates reach the colon on a daily basis⁽⁴⁷⁾, including resistant starch, NSP, plant cell wall polysaccharides and non-digestible oligosaccharides^(47–49). Some dietary proteins (e.g. collagen and elastin) as well as various secondary plant metabolites (e.g. polyphenolic substances) can also reach the large intestine and may undergo bacterial transformations^(50,51).

Alternative substrates can give rise to different products due to fermentation via different metabolic processes, while the same substrate can be metabolised by different pathways depending on the rate of supply or the physiology and environment of the bacterial cell⁽⁵⁰⁾. Changes in the composition of the gut microbiota in response to dietary intake occur because different bacterial species are better equipped (genetically) to utilise different substrates⁽⁴⁹⁾. Generally, bacteria favour carbohydrates as primary energy sources if they are available⁽⁵²⁾. Metagenomic sequencing of the intestinal microbiota has identified a large group of carbohydrate-active enzymes⁽⁵³⁾. While certain species, particularly those of the phylum Bacteroidetes, possess large numbers of genes encoding carbohydrate-active enzymes, which allows them to switch between different energy sources, other groups encode fewer carbohydrate-active enzymes and are noticeably more specialised⁽⁴⁷⁾. Dietary supplementation with prebiotics such as inulin and fructo-oligosaccharides can promote the growth of specific groups of bacteria, including bifidobacteria^(54–56). A recent study has demonstrated rapid and reversible changes in the relative abundance of specific dominant bacterial groups after changes in the major type of non-digestible carbohydrate (i.e. resistant starch, NSP or reduced-carbohydrate diet). There were profound inter-individual differences in the response of the microbial community to dietary change due to inter-individual differences in the initial microbial composition; this suggests that dietary advice on the consumption of non-digestible carbohydrates may need to be personalised in the future⁽⁵⁷⁾.

Saccharolytic bacterial fermentation mainly takes place in the proximal colon (due to greater availability of fermentable carbohydrates)⁽⁵⁸⁾ and may result in the production of SCFA⁽⁵¹⁾, the type and levels of which depend on the source and quantity of carbohydrates available and the microbiota present⁽⁴⁹⁾. SCFA are energy sources for the colonic epithelium, and butyrate, in particular, exerts important effects on cell differentiation and gut health⁽⁵⁸⁾. Proteolytic fermentation generally takes place in the distal colon (where fermentable carbohydrates become depleted)⁽⁵⁸⁾ and results in the production of SCFA in addition to ammonia, amines, phenols, thiols and indoles⁽⁵¹⁾.

Early studies comparing dietary patterns (e.g. 'Japanese' *v.* 'Western') or examining the impact of changing the proportions of food categories on the intestinal microbiota have found only moderate effects involving a few genera^(59–61). These studies relied on culture-based techniques and were, therefore, limited in their ability to detect changes in the fine detail of the composition of the gut microbiota. More recent studies have employed culture-independent approaches and have further elucidated the role of diet in the determination of the composition of the intestinal microbiota in humans (Table 1).

In a landmark study, De Filippo *et al.*⁽⁶²⁾ compared the faecal microbiota of European children (consuming a 'Western' diet) with that of children in the African state of Burkina Faso (consuming a plant-rich, 'rural' diet, high in fibre content). The Burkina Faso children had a lower abundance of bacteria of the phylum Firmicutes and a higher abundance of those of the phylum Bacteroidetes (mainly *Prevotella* and *Xylanibacter*) in their faecal microbiota compared with the European children, who had higher levels of Enterobacteriaceae. *Prevotella* and *Xylanibacter*, which contain genes for cellulose and xylan hydrolysis, were associated with increased levels of faecal SCFA. The authors postulated that the gut microbiota co-evolved with the plant-rich diet of the Burkina Faso children, allowing them to maximise energy extraction from dietary fibre while also protecting them from inflammation and non-infectious intestinal diseases.

Similar dietary associations have been found in a study linking the dietary patterns of American adults with gut microbial enterotypes, dominated by *Bacteroides* or *Prevotella*. Wu *et al.*⁽¹⁷⁾ found that the *Bacteroides* enterotype was positively associated with protein and animal fat, whereas the *Prevotella* enterotype was associated with a diet high in carbohydrates and low in meat and dairy products.

Vegetarianism has also been shown to alter the composition of the intestinal microbiota^(63–65). The higher intakes of carbohydrate and fibre associated with this dietary practice result in the production of SCFA by microbes, which lowers the intestinal pH, preventing the growth of potentially pathogenic bacteria such as *E. coli* and other members of Enterobacteriaceae spp.⁽⁶⁶⁾. Indeed, Zimmer *et al.*⁽⁶⁶⁾ demonstrated that subjects consuming a vegan or vegetarian diet had lower stool pH than controls and that total counts of culturable *Bacteroides* spp., *Bifidobacterium* spp., *E. coli* and Enterobacteriaceae spp. were significantly lower in

vegan samples than in the controls. A vegetarian diet has also been shown to decrease the amount and change the diversity of *Clostridium* cluster IV and *Clostridium* rRNA clusters XIVa and XVII^(63,64).

It has recently been reported that diverse dietary patterns are responsible for the variation in the compositions of the gut microbiota observed between community-dwelling elderly subjects and subjects in long-term residential care. The diet of community-dwelling individuals was typically more diverse with low-to-moderate fat and high fibre intakes, whereas that of subjects in long-term residential care was less diverse with moderate-to-high fat and low-to-moderate fibre intakes. Those in long-term care had a less diverse microbiota with a higher proportion of bacteria of the phylum Bacteroidetes, while community-dwelling subjects had a more diverse microbiota with a higher proportion of bacteria of the phylum Firmicutes. Community-dwelling subjects had a higher abundance of bacteria of the genus *Prevotella*, supporting the association between *Prevotella* and a diet high in carbohydrates as observed in the Burkina Faso children and American adults. *Coprococcus* and *Roseburia* were also more abundant in the faecal microbiota of community-dwelling subjects, whereas *Parabacteroides*, *Eubacterium*, *Anaerotruncus*, *Lactonifactor* and *Coprobacillus* were more abundant in subjects in long-term care. For subjects in long-term care, both the faecal microbiota and diet were associated with the duration of stay, with subjects residing for more than 1 year having diet and microbiota that were furthest separated from those of community-dwelling subjects compared with recently admitted subjects. Interestingly, the major trends in the microbiota that separated the community-dwelling elderly from the elderly in long-term care were associated with changes in frailty, inflammation and other clinical markers and hence indicate a role for diet-driven microbial composition alterations in health among the elderly⁽²⁰⁾.

Changes in the abundance of the gut microbiota of (humanised germ-free (GF)) mice have been analysed after the mice were switched from a diet low in fat and rich in plant polysaccharides to a 'Western' diet high in fat and sugar and low in plant polysaccharides. After just a single day, mice on the 'Western' diet displayed an increased abundance of bacteria of the phylum Firmicutes and a decreased abundance of those of the phylum Bacteroidetes⁽⁶⁷⁾. Hildebrandt *et al.*⁽⁶⁸⁾ also found distinctive changes in the abundance of the gut microbiota of mice following a switch from a standard chow to a high-fat diet, which was associated with a proportional decrease in the abundance of bacteria of the phylum Bacteroidetes and an increase in that of both Firmicutes and *Proteobacteria*.

Faith *et al.*⁽⁶⁹⁾ developed a statistical model for predicting how a change in diet would alter the abundance of particular species of the gut microbiota. A model community of ten genome-sequenced human intestinal bacteria (including *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, *Bacteroides caccae*, *E. coli*, *Desulfovibrio piger*, *Collinsella aerofaciens*, *Clostridium symbiosum*, *Blautia hydrogenotrophica*, *Eubacterium rectale* and *Marvinbryantia formatexigens*) was introduced into GF mice, and the composition of the intestinal



Table 1. Associations of the human intestinal microbiota with habitual dietary patterns or interventions

Authors	Methods	Study design	Subjects	Diets/nutrients	Microbial response
Claesson <i>et al.</i> ⁽²⁰⁾	16 s rDNA sequencing	Cross-sectional	178 elderly subjects (age 64–102 years) – community, day hospital, rehabilitation and long-stay subjects	'Community' diet – diverse with low–moderate fat/high fibre 'Long-stay' diet – reduced diversity with moderate–high fat/low–moderate fibre	↑ Diversity ↑ Firmicutes ↑ <i>Coprococcus</i> , <i>Roseburia</i> ↓ Diversity ↑ Bacteroidetes ↑ <i>Parabacteroides</i> , <i>Eubacterium</i> , <i>Anaerotruncus</i> , <i>Lactonifactor</i> and <i>Coprobacillus</i>
De Filippo <i>et al.</i> ⁽⁶²⁾	16 s rDNA sequencing and biochemical analysis	Cross-sectional	Twenty-nine children (1–6 years) – African children from Burkina Faso (<i>n</i> 14) and European children from Florence, Italy (<i>n</i> 15)	'Western' diet – high fat/protein/sugar and low fibre 'Rural' diet – low fat/protein and high fibre	↑ Firmicutes ↑ Enterobacteriaceae ↑ Bacteroidetes exclusively present: <i>Prevotella</i> , <i>Xylanibacter</i> , <i>Butyrivibrio</i> and <i>Treponema</i> ↑ SCFA
De Palma <i>et al.</i> ⁽⁷⁰⁾	FISH and qPCR	Feeding (1 month)	Ten healthy subjects (mean age 30.3 years)	Gluten-free diet (reduced polysaccharide)	↓ <i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Clostridium lituseburensis</i> and <i>Faecalibacterium prausnitzii</i> ↑ Enterobacteriaceae and <i>Escherichia coli</i> ↓ <i>Clostridium</i> cluster XIVa ↓ <i>Roseburia</i> – <i>Eubacterium rectale</i> ↓ butyryl-CoA CoA-transferase gene
Kabeerdoss <i>et al.</i> ⁽⁶⁵⁾	qPCR	Cross-sectional	Fifty-six healthy female subjects (age 18–27 years); thirty-two vegetarians and twenty-four omnivores	Vegetarian diet	↑ Bacterial DNA tendency for ↓ <i>Clostridium</i> cluster IV and ↑ <i>Bacteroides</i> (but not significant)
Liszt <i>et al.</i> ⁽⁶⁴⁾	qPCR and PCR-DGGE	Cross-sectional	Twenty-nine healthy subjects (age 19–34 years) – fifteen vegetarians and fourteen omnivores	Vegetarian diet	Associated with KEGG orthology groups Associated with bacterial OTU content
Muegge <i>et al.</i> ⁽⁷¹⁾	16 s rDNA sequencing and shotgun metagenomics	Cross-sectional	Eighteen lean subjects (mean age 59.6 years) – members of a Calorie Restriction Society	Proteins Insoluble dietary fibre	↔ Phylum level ↑ <i>Ruminococcus bromii</i> and <i>E. rectale</i> ↑ <i>Ruminococcaceae</i> ↑ <i>Oscillibacter valericigenes</i> ↑ Firmicutes bacteria related to <i>Roseburia</i> and <i>E. rectale</i>
Walker <i>et al.</i> ⁽⁵⁷⁾	16 s rDNA sequencing and qPCR	Randomised cross-over (3-week intervention)	Fourteen overweight male subjects (age 27–73 years)	Diet high in resistant starch (type III) Reduced-carbohydrate diet (weight-loss diet)	↔ Phylum level ↓ <i>Collinsella aerofaciens</i> ↑ <i>O. valericigenes</i> ↓ Firmicutes bacteria related to <i>Roseburia</i> and <i>E. rectale</i> ↓ Bacteroidetes, <i>Actinobacteria</i> ↓ Firmicutes, <i>Proteobacteria</i> ↓ Bacteroidetes, <i>Actinobacteria</i> ↑ Firmicutes, <i>Proteobacteria</i>
Wu <i>et al.</i> ⁽¹⁷⁾	16 s rDNA sequencing and shotgun metagenomics	Cross-sectional	Ninety-eight healthy subjects (age 18–40 years)	Fat Fibre Animal fat and protein Carbohydrates	Positively associated with <i>Bacteroides</i> enterotype Positively associated with <i>Prevotella</i> enterotype
Wu <i>et al.</i> ⁽¹⁷⁾	16 s rDNA sequencing and shotgun metagenomics	Controlled feeding (10 d intervention)	Ten subjects having <i>Bacteroides</i> enterotype (high fat/protein)	Low-fat/high-fibre diet or high-fat/low-fibre diet	Changes in the composition of microbiome detectable within 24 h of consuming diet; no stable switch in enterotype after 10 d
Zimmer <i>et al.</i> ⁽⁶⁶⁾	Culture-based methods	Cross-sectional	295 healthy subjects – 144 vegetarians, 105 vegans and forty-six controls	Vegetarian diet Vegan diet	↓ Stool pH ↓ Stool pH ↓ <i>Bacteroides</i> spp., <i>Bifidobacterium</i> spp., <i>E. coli</i> and Enterobacteriaceae spp.

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↑, Increased; ↓, decreased; FISH, fluorescent *in situ* hybridisation; qPCR, quantitative real-time PCR; DGGE, denaturing gradient gel electrophoresis; KEGG, Kyoto Encyclopedia of Genes and Genomes; OTU, operational taxonomic unit; ↔, no change.

community as the mice consumed different proportions of protein (casein), fat ('corn' oil), polysaccharide ('cornstarch') and sugar (sucrose) was monitored. Each mouse was fed a randomly selected diet with diet switches occurring every 2 weeks. Steady-state levels of community members were achieved within 4 d of a diet change. Notably, the total DNA yield per faecal pellet increased as the amount of casein in the host diet was increased. In addition, changes in species abundance as a function of changes in casein concentration in the host diet were apparent for all the ten species; the abundance of seven species was positively correlated with casein concentration, whereas that of the remaining three species (*E. rectale*, *M. formatexigens* and *D. piger*) was negatively correlated with casein concentration. Indeed, inspection of the most highly expressed genes of *E. rectale* and *M. formatexigens* indicated that they focused on carbohydrate catabolism, whereas *D. piger* can use only a restricted number of substrates (e.g. lactate, H₂ and succinate). In a follow-up experiment involving diets containing various mixtures of puréed human baby foods (i.e. foods more typically consumed in human diets), changes in species abundance that were a function of diet ingredients (e.g. apple, beef, chicken, oat, pea, peach, rice and sweet potato) were found. For example, *B. ovatus* increased in absolute abundance with an increased concentration of oats in the diet, whereas most of the bacterial species responded to multiple ingredients.

Although these results from animal studies are interesting, it can be difficult to apply these findings to humans due to the artificial nature of these experiments compared with natural human microbiota and food consumption patterns. Only a limited number of human clinical trials have assessed the effects of dietary pattern changes on the intestinal microbiota^(17,57,70,71). In a controlled-feeding study with ten individuals (*Bacteroides* enterotype), Wu *et al.*⁽¹⁷⁾ found that the composition of the microbiome changed detectably within 24 h of consuming a high-fat/low-fibre or low-fat/high-fibre diet, showing the rapid effect that diet can have on the intestinal microbiota. However, enterotype identity remained constant, with no stable changes in the composition of the *Prevotella* enterotype, indicating that alternative enterotype states are associated with long-term diet intake.

In overweight men, supplementation of the diet with resistant starch increased the faecal levels of *E. rectale* and *Ruminococcus bromii*, which correlated with the fermentation of fibre. However, the inter-individual variation in the responses of the microbiota to resistant starch indicates that dietary interventions may need to be personalised⁽⁵⁷⁾.

In another study, a gluten-free diet intervention featuring a reduction in overall polysaccharide intake led to reductions in gut bacterial populations such as *Bifidobacterium*, *Clostridium lituseburense* and *Faecalibacterium prausnitzii* and proportional increases in the abundance of *E. coli* and total Enterobacteriaceae in healthy volunteers^(70,72).

Based on the available data, differences in the compositions of the gastrointestinal microbiota are demonstrable between groups of people living on different diets. These diet-associated changes in composition can lead to changes in the metabolic activity of the intestinal microbiota, which, in turn, may

provoke changes in inflammatory and immune responses. Although attempts to change the composition of the intestinal microbiota by varying the diet have been successful in mice, there is a relative paucity of human dietary intervention studies, and those available are small in sample size and have been conducted over a short period of time. Moreover, mechanisms that link dietary changes to microbial composition alterations remain poorly defined and need to be investigated further. Large, well-controlled trials are also required to determine the impact of altering long-term dietary patterns on the human intestinal microbiota and to elucidate the implications of the key population changes for health and disease.

Antibiotics

Antibiotic treatment has been shown^(42,73–75) to dramatically disturb the composition of the faecal microbiota in humans. Palmer *et al.*⁽⁷⁶⁾ reported changes in the density or composition of the intestinal microbiota in infants following antibiotic treatment. Striking changes have been found in some cases, even to a point where the faecal microbiota was undetectable. As there is considerable inter-individual variability in the composition of the microbiota among humans⁽⁴²⁾, it has been suggested that the impact of antibiotics is best assessed on an individual basis⁽⁷⁷⁾. In general, antibiotic treatment leads to a decrease in the diversity of the microbiota⁽⁷⁸⁾. Nonetheless, the community is quite resilient and can resemble the pre-treatment state in a matter of days or weeks^(42,79,80). However, a number of other studies have shown that microbial composition alterations following antibiotic administration can often persist for a long time period following withdrawal of the treatment, with some members of the microbial community failing to return to pre-treatment levels and these may even be lost from the community indefinitely^(77,79,81–83). Disruption of the microbiota by antibiotics can also affect the metabolic activity of the bacterial community in the gut. Antibiotic treatment in mice has been shown to drastically alter the intestinal metabolome by affecting host metabolic pathways such as sugar, nucleotide and fatty acid metabolism as well as bile acid, eicosanoid and steroid hormone synthesis coding capacity⁽⁸⁴⁾.

The effect of antibiotics on the intestinal microbiota in infancy is of particular concern. Recent reports have demonstrated that short-term parenteral antibiotic treatment of neonates causes significant alterations in the composition of the gut microbiota including a disturbance of the expected colonisation pattern of bifidobacteria^(85,86). Colonisation of the intestine early in life has an important role in directing immune system development, and antibiotic use may increase the risk of atopy and allergic asthma by reducing the protective effect of microbial exposure^(87,88). In a large, multi-centre study, Foliaki *et al.*⁽⁸⁸⁾ found an association between antibiotic use in the first year of life and symptoms of asthma, rhinoconjunctivitis and eczema in children aged 6 and 7 years.

The impact of antibiotic use on the intestinal bacteria of the elderly is also of interest, since, compared with younger adults, cohorts of elderly populations are typically administered a complex array of medications, including

antibiotics. Antibiotic treatment in hospitalised elderly subjects has been shown to increase the intestinal abundance of proteolytic bacteria⁽⁷³⁾, to reduce overall bacterial numbers and, in some subjects, to completely eliminate certain bacterial communities⁽⁴⁶⁾. A more recent study has found that antibiotic treatment led to a decrease in the taxonomic richness, diversity and evenness of the intestinal community in elderly subjects, although the magnitude of the changes and the taxa affected were different between subjects. Moreover, the overall community structure was restored within 4 weeks of treatment⁽⁴²⁾.

One of the best-known complications arising following antibiotic therapy is antibiotic-associated diarrhoea (AAD)⁽⁸⁹⁾. A number of mechanisms underlie the development of AAD. Antibiotic therapy can disturb the natural microbiota in the GIT, which may result in the pathological overgrowth of *C. difficile*, and it may also disturb the metabolism of carbohydrates, giving rise to malabsorption of osmotically active particles^(75,90). Young & Schmidt⁽⁷⁵⁾ found that in a patient who developed AAD, antibiotic administration was associated with distinct changes in the diversity of the gut microbiota, including a decrease in the prevalence of butyrate-producing bacteria. Following discontinuation of antibiotic treatment, resolution of diarrhoea was accompanied by a reversal of these changes. This provided evidence linking changes in the community structure of the gastrointestinal bacteria with the development of AAD.

The impact of antibiotic use in the short and long terms needs to be investigated further. Longitudinal type studies rather than cross-sectional studies will allow more direct testing of questions regarding the influence of antibiotic use on the development of allergy and gastrointestinal diseases, particularly in early life.

Disease

Inflammatory bowel diseases. Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic intestinal disorders whose aetiology is unclear. However, an abnormal immune response against luminal antigens, such as dietary factors and/or bacteria, may be involved^(91,92). CD can affect any part of the GIT, although the lower ileum and colon are most commonly involved⁽⁹³⁾. It is characterised by discontinuous inflammation of the epithelial lining and deep ulcers. UC affects only the colon and rectum and is characterised by continuous mucosal inflammation and superficial ulcers⁽⁹⁴⁾. The clinical symptoms of IBD include abdominal pain, diarrhoea, rectal bleeding, malaise and weight loss⁽⁹³⁾.

Numerous studies have compared the compositions of the intestinal microbiota of IBD patients and healthy individuals, and it appears that the dominant microbiota differs between the two groups (reviewed in Ojetti *et al.*⁽⁹¹⁾, Gerritsen *et al.*⁽⁹⁴⁾, Dickved *et al.*⁽⁹⁵⁾ and Shanahan⁽⁹⁶⁾). Similarly, the dominant microbiota in patients with UC differs from that in patients with CD^(91,94). However, some changes in the composition of the microbiota are similar between the UC and CD patients⁽⁹⁵⁾.

Although the phylum-level changes observed in IBD patients have not been consistent always, in general, an overall decrease in microbial diversity and stability of the intestinal microbiota has been observed in IBD patients^(94,95). A decrease in the abundance of specific members of the phylum Firmicutes has been reported, which in some cases coincided with an increase in the abundance of those of the phylum Bacteroidetes and that of facultative anaerobes such as Enterobacteriaceae⁽⁹⁴⁾. Moreover, increased numbers of *E. coli*, some of which may be pathogenic, have been observed in IBD patients⁽⁹⁵⁾. Increased detection of *C. difficile* in relapse and remission of both forms of IBD has been observed⁽⁹⁶⁾. Other reports have described alterations in the abundance of Bacteroidetes spp., proteobacteria, bifidobacteria and lactobacilli, but results have been inconsistent⁽⁹⁵⁾.

With regard to CD, a number of consistent observations have been reported⁽⁹⁶⁾. These include increased mucosal bacterial counts, increased levels of adherent-invasive *E. coli* and increased levels of *Mycobacterium avium* subsp. *paratuberculosis*. Furthermore, a reduced number of bacteria in the *Clostridium leptum* group, including *F. prausnitzii*, have been observed in CD patients⁽⁹⁷⁾. In fact, *F. prausnitzii* has even been proposed as a potential probiotic for counterbalancing dysbiosis in CD⁽⁹⁷⁾. For UC, a reduced presence of the *Clostridium coccoides* group has been described, but no specific members of this group have been reported to be associated with the disease yet⁽⁹⁵⁾.

Although marked alterations occur in the composition of the gut microbiota of IBD patients, it is unclear whether these shifts cause the disease or whether they arise due to the changes in the gut environment that result from the disease. Indeed, most of the studies carried out to date have reported associations between the microbiota and IBD only after the IBD phenotype has emerged, which does not allow one to answer the important question of what came first – IBD or a change in the microbiome. More long-term longitudinal studies are needed to examine the progression of diseases and to typify the taxonomic and functional composition changes of the microbiome that lead to or may even define IBD.

Irritable bowel syndrome. Irritable bowel syndrome (IBS) is a common, debilitating gastrointestinal disorder characterised by abdominal pain, bloating and disturbances in bowel function^(98–100). IBS can present as diarrhoea-predominant IBS, constipation-predominant IBS or mixed-bowel-habit IBS⁽¹⁰¹⁾.

IBS can be difficult to diagnose due to the lack of a biological or pathogenic marker^(98,99). Although the pathophysiology of IBS is still not well understood, several factors are thought to play a role. These include malfermentation of food ingredients, altered microbial composition, intestinal motor and sensory dysfunction, immune mechanisms, psychological factors and brain–gut axis dysregulation^(99,102,103). Considerable evidence suggests that factors that disturb the gut microbiota, such as gastroenteritis, may contribute to the development of IBS⁽¹⁰⁴⁾.

Differences in the compositions of the intestinal microbiota between IBS patients and healthy controls (HC) have mostly

been studied using faecal material. Mättö *et al.*⁽¹⁰⁵⁾, using culture-based techniques, observed slightly higher numbers of culturable coliforms and an increased aerobic:anaerobe ratio in IBS subjects relative to HC. Moreover, PCR-denaturing gradient gel electrophoresis has revealed more temporal instability in the predominant bacterial population of IBS subjects than in controls, and IBS subjects had more *Clostridium* spp. and less *Eubacterium* spp. amplicons. However, the researchers did not control for antibiotic use, which may have contributed to the apparent temporal instability observed⁽¹⁰⁵⁾. In a subsequent study, which targeted the clostridial groups in IBS, it has been reported that a similar instability existed⁽¹⁰⁶⁾. In addition, a study employing denaturing gradient gel electrophoresis techniques has found that there was significantly more variation in the gut microbiota of healthy volunteers than in that of IBS patients⁽¹⁰⁷⁾.

Jeffery *et al.*⁽¹⁰⁰⁾ described a detailed analysis of the faecal microbiota in a cohort of well-characterised IBS patients and control subjects and found no uniform change in the composition of the microbiota in IBS patients. However, analysis of the microbial populations revealed distinct clusters, one of which showed normal-like microbial composition compared with HC samples. The other IBS samples were characterised by an increased Firmicutes: Bacteroidetes ratio. In addition, analysis of the IBS microbiota and separate analyses of the two subgroups have shown microbial associations with colonic transit time, satiety, bloating, rectal pain threshold and depression⁽¹⁰⁰⁾. Significantly, IBS subjects with a microbiota similar to that of the matched HC displayed higher anxiety and depression scores, suggesting a non-intestinal or at least a non-microbiota aetiology for IBS in this subgroup.

A more recent study has shown intestinal dysbiosis in diarrhoea-predominant IBS patients compared with HC. A significant increase in the abundance of unclassified Enterobacteriaceae members and significant reductions in that of the members of the *Faecalibacterium* genus have been found in IBS patients compared with controls. Furthermore, *Enterococcus*, *Fusobacterium*, *Pediococcus*, unclassified Lactobacillaceae and *Veillonella* species have been found in IBS patients, but reported to be below detection limits in HC⁽¹⁰⁸⁾.

The studies described above demonstrate that the intestinal microbiota of patients with IBS can differ from that of healthy individuals. Nonetheless, it is not yet possible to be certain (as in IBD discussed above) whether the alterations in the composition of intestinal microflora observed in IBS patients are the cause of IBS or simply a result of the disrupted gut motility or other physiological features of IBS. More studies are needed to clarify whether the microbiota has a causal role in the initiation and/or progression of IBS.

Obesity. Some of the earliest evidence showing the role of the gut microbiota in the regulation of fat storage has been demonstrated in animal models. A pioneering study by Bäckhed *et al.*⁽¹⁰⁹⁾ has found that GF mice were leaner than their conventional counterparts and colonisation with an intestinal microbiota resulted in a significant increase in body fat content despite lower food consumption in the

colonised animals. A subsequent study has found that GF mice were protected against obesity following consumption of a Western-style, high-fat, sugar-rich diet⁽¹¹⁰⁾. In addition, the colonisation of GF mice with an 'obese microbiota' (i.e. from an obese animal) has been reported to lead to greater increases in total body fat compared with GF mice colonised with a 'lean microbiota'⁽¹¹¹⁾, indicating that the obese microbiota has an increased capacity to harvest energy from the diet.

It has been suggested that inflammation⁽¹¹²⁾ and alterations in host gene expression⁽¹¹⁰⁾ are other mechanisms by which the gut microbiota may influence the host. Obesity and its related metabolic disorder, type 2 diabetes, are generally associated with chronic low-grade inflammation⁽¹¹³⁾. Lipopolysaccharide (LPS), a highly pro-inflammatory component, is a possible initiator of metabolic impairment⁽¹¹²⁾. Plasma LPS levels increase with higher fat intake in both mice⁽¹¹²⁾ and humans⁽¹¹⁴⁾, and the direct infusion of LPS mimics the physiological effects of a high-fat diet in mice⁽¹¹²⁾. It has been hypothesised that LPS is taken up with dietary fats in chylomicrons⁽¹¹⁵⁾ or that LPS reaches the circulation because the gut is more permeable in obese mice due to the disruption of tight junction proteins^(116,117). A review on this topic has been described recently⁽¹¹⁸⁾.

Studies have also linked alterations in the composition of the intestinal microbiota to obesity^(119–122). An increased ratio of Firmicutes:Bacteroidetes has been observed in genetically obese mice (ob/ob)⁽¹²⁰⁾ as well as obese humans^(23,121). However, a number of other studies have failed to confirm these findings and have shown variable patterns in phylum-level changes measured in the composition of the microbiota of obese humans^(119,123,124).

Although it is clear from the studies described above that the gut microbiota is likely to play some role in obesity and metabolic disease, it is difficult to draw definite conclusions on the importance of particular bacterial groups. Further well-designed, large clinical studies are required to identify microbiota-related biomarkers of risk for obesity and metabolic dysregulation.

Manipulation of intestinal microbiota

Probiotics

The term 'probiotic', a word derived from Greek and meaning 'for life'⁽¹⁰³⁾, has been defined as 'live microorganisms which when administered in adequate amount confer a health benefit on the host'⁽¹²⁵⁾. Some probiotic products contain a single strain, while others contain a mixture of several species of bacteria or fungi. The most-studied and commonly used organisms in probiotic preparations are lactobacilli and bifidobacteria.

One of the original concepts associated with probiotics was that their consumption would alter the composition of the intestinal microbiota from a possibly harmful one towards a microbiota that would benefit the host (i.e. replace 'bad' bacteria with 'good' bacteria)⁽¹²⁶⁾. This was a rather simplistic theory, and it was not based on a full understanding of the



complexity of the intestinal microbiota. It has since been suggested⁽¹²⁷⁾ that too much emphasis should be placed not on the potential change in the composition of the microbiota, but rather on the inherent health benefits conferred by the probiotics themselves. Indeed, for some probiotic effects (e.g. immune modulation), it may not be necessary to achieve a measurable modification of the composition of the intestinal microbiota. In recent times, two main motives have emerged for the use of probiotics. The first is the use of probiotics by healthy subjects to maintain a healthy state and decrease the risk of illness. The second is the use of probiotics as a treatment/therapeutic modality targeted at particular diseases.

There are a variety of proposed health effects (both direct and indirect) of probiotics, which have been reviewed extensively^(128–130). However, the subtleties of the positive effects of probiotics can only be fully appreciated following a meta-analysis. Some of the most robust clinical data are confined to the preventive and therapeutic effects of probiotic strains on diarrhoeal illness^(131–134). A number of beneficial effects of probiotics dealing with intestinal health have been evaluated in Cochrane reviews^(132,134–136). These and other meta-analyses have demonstrated the efficacy of probiotics in the prevention and treatment of AAD^(131,133,136), acute infectious diarrhoea⁽¹³²⁾ and persistent diarrhoea in children⁽¹³⁴⁾. Evidence is also accumulating from well-conducted clinical

studies on the efficacy of probiotics in the prevention and reduction of the severity of necrotising enterocolitis in premature infants and those with very low birth weight^(135,137,138). Probiotics have also yielded promising improvements in the prevention and treatment of IBD (UC and CD)⁽⁹²⁾ and IBS⁽¹⁰³⁾. However, it must be noted that these meta-analyses have their own limitations. The clinical and methodological heterogeneity between studies as well as the differences in probiotic type, delivery method (yogurt *v.* capsule) and dosage makes comparisons difficult. Indeed, no two different probiotics are likely to be functionally the same, and therefore performing meta-analyses based on studies involving different strains, species and even genera is inherently questionable. Different strains may have vastly different effects, and hence no ideal probiotic strain for any of the above-mentioned conditions has been identified, despite continuing advances in this area.

Although there is no single mechanism of action for probiotics, there are a number of common mechanisms by which probiotics might influence the intestinal microbiota (Fig. 2)⁽¹³⁹⁾. However, it is likely that the mechanism of action of probiotics is multifactorial and strain specific⁽¹⁴⁰⁾.

While there are extensive scientific and clinical portfolios associated with (specific strains of) probiotics, the European Food Safety Authority is yet to approve health claims for a

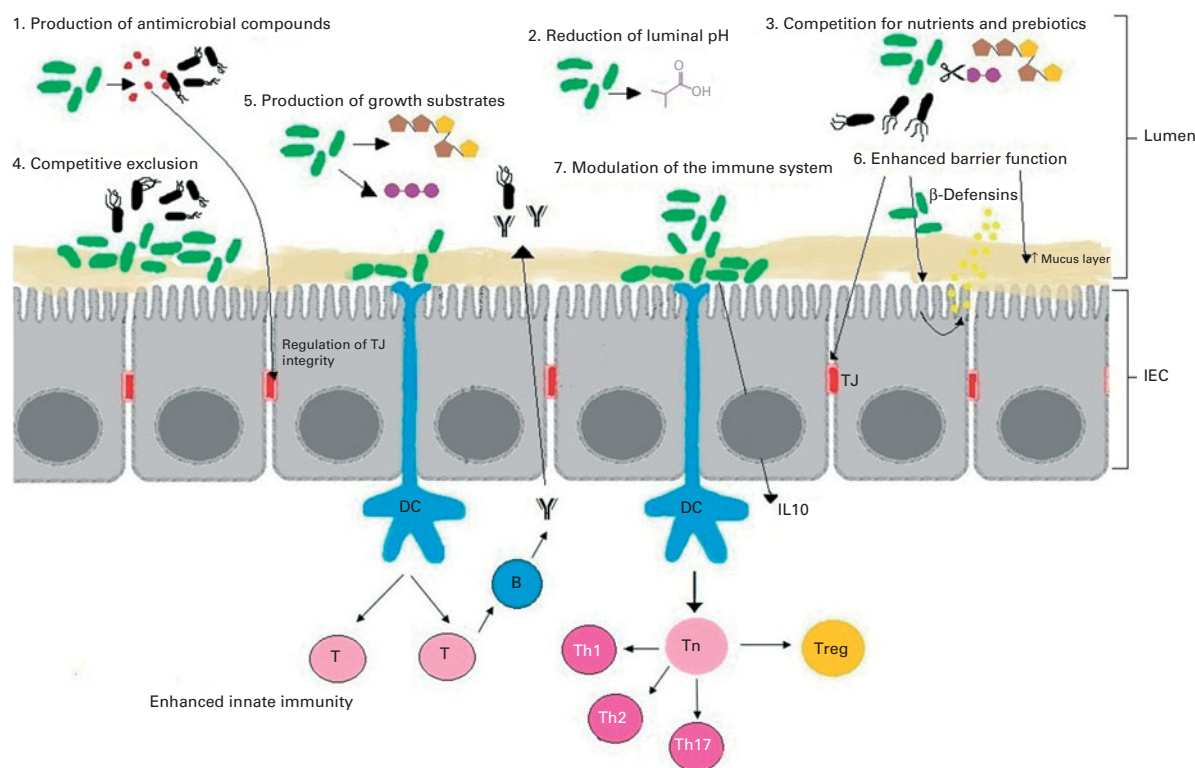


Fig. 2. Schematic diagram illustrating the selected mechanisms by which probiotic bacteria may influence the intestinal microbiota and/or induce beneficial host responses: (1) production of antimicrobial compounds (e.g. bacteriocins); (2) reduction of luminal pH through the production of SCFA; (3) competition with pathogens for nutrients and prebiotics; (4) competitive exclusion of pathogens for adhesion to epithelial cells; (5) production of growth substrates (e.g. vitamins, SCFA and exopolysaccharide); (6) enhanced intestinal barrier function (e.g. increased mucus and β -defensin secretion and/or modulation of cytoskeletal and tight junction protein phosphorylation); (7) modulation of immune response. IEC, intestinal epithelial cells; DC, dendritic cells; TJ, tight junction (modified from O'Toole & Cooney⁽¹³⁹⁾). B, B cells; T, T cells; Th, T helper cells; Tn, naive T cells; Treg, regulatory T cells.

single probiotic (there have been 120 negative opinions on probiotic claims through February 2011)⁽¹⁴¹⁾. Indeed, regulators are almost applying pharmaceutical standards to the use of probiotics. As has been mentioned already, there are two main uses for probiotics: (1) probiotics as a 'food-for-health' product and (2) probiotics as a therapeutic modality for illness. The first example is clearly the one where the food industry is focusing its resources – that is, keeping healthy people healthy. One may ask whether it is appropriate for the regulators to apply pharmaceutical industry standards of proof for probiotics that are largely intended to be given to healthy people? Moreover, unlike pharmaceutical drugs with a single active entity, probiotics encompass hundreds of different strains and hundreds of different surface molecules and possibly metabolites that may be responsible for the 'probiotic' effect. Certainly, this is a regulatory challenge that is yet to be resolved. As a scientist interested in exploring the functions of probiotics, one has to be in favour of stringent regulations, and the challenge is now firmly on commercial probiotic purveyors to generate high-quality scientific data that will allow them to make health claims. However, one can also conclude that trying to apply concepts and standards the same as those employed for pharmaceuticals may not be appropriate for probiotics. Scientists, companies and regulators need to address these issues. Otherwise, credibility with the consumer, interest shown by the food industry and ultimately scientific research will be seriously damaged.

Recently, there has been interest in faecal transplantation as an alternative approach for the manipulation of the intestinal microbiota. Indeed, evidence for its use as a treatment for gastrointestinal illness (including pseudomembranous colitis, *C. difficile*-associated diarrhoea, antibiotic-associated diarrhoea, IBS and IBD) is rapidly accumulating and has been reviewed recently^(142–144). Faecal transplantation as a treatment modality remains a controversial issue, and the evidence available for its efficacy is limited. Nonetheless, this therapy holds great promise and further studies are necessary to explore this potential.

Probiotics: dead or alive? It has been proposed that the minimum therapeutic dose for probiotics is 10^8 – 10^9 viable cells per d⁽¹⁴⁵⁾. However, live cells in probiotic products will inevitably lose viability and the actual products will contain varying proportions of populations of viable-to-non-viable/dead cells⁽¹⁴⁶⁾. There may be further losses of viability of the organisms on passage through the relatively hostile environment of the stomach and small intestine⁽¹⁴⁷⁾. Concerns have also been raised that the administration of live microorganisms may not be appropriate for some population groups (e.g. premature infants and immunocompromised individuals), as they may translocate to the locally draining tissues, thereby causing bacteraemia and sepsis^(148,149).

Therefore, an area of related ongoing debate is whether or not non-viable forms of beneficial bacterial strains have a role in the conferment of benefits on the host. Indeed, a considerable amount of published scientific evidence indicates that inactivated microbes may positively affect health by influencing the host immune system (reviewed in Adams⁽¹⁴⁷⁾, Kataria *et al.*⁽¹⁴⁸⁾ and Taverniti & Guglielmetti⁽¹⁵⁰⁾). The ability of

bacterial cells to potentially interact with the host, independent of viability, is based on the capacity of human cells to recognise specific bacterial components or products, leading to responses that commonly involve the mucosa-associated lymphoid tissue and, therefore, the immune system⁽¹⁴⁷⁾. Some studies have proposed that the immunomodulatory effects exerted by non-viable probiotics may be due to their immunostimulatory DNA, cell wall components, peptidoglycan, intra- and extracellular polysaccharide products and cell-free extracts^(151–154).

A number of studies have evaluated the immunomodulatory effect of the probiotic *Lactobacillus rhamnosus* GG, in both live and dead (inactivated) forms. Heat-killed or UV-inactivated *L. rhamnosus* GG may reduce inflammation by decreasing experimentally induced IL8 production by epithelial Caco-2 cells^(149,155). In the absence of induction, high doses of live *L. rhamnosus* GG actually induce IL8 production, while the heat-killed agent causes only a slight increase in IL8 production, meaning that it has a lower potential to cause inflammation itself⁽¹⁵⁵⁾, thus indicating that the heat-killed agent may be a safer alternative. A similar response has been demonstrated in an animal model, in which both live and heat-killed *L. rhamnosus* GG reduced the levels of LPS-induced pro-inflammatory mediators and up-regulated the levels of anti-inflammatory mediators in intestinal tissue in rats⁽¹⁵⁶⁾. Similarly, heat-killed *Lactobacillus* strains have been found to induce TNF α secretion in mouse splenic mononuclear cells to various degrees⁽¹⁵⁷⁾. Furthermore, the purified surface glycolipid lipoteichoic acid, which is a major component of the cell wall of lactobacilli, activated macrophages through toll-like receptor 2 (TLR2) in a strain-specific manner. It has even been suggested that the immense structural diversity in lipoteichoic acid derived from different bacteria may induce a variety of immunoregulatory effects⁽¹⁵⁸⁾.

The immunoregulatory potential of exopolysaccharide (EPS) has also been investigated^(158–162). It has been shown that *in vitro* levels of pro-inflammatory cytokines are highly elevated upon exposure of mouse splenocytes to cells of EPS-deficient *Bifidobacterium breve* co-cultures, whereas exposure to an EPS-producing strain has been shown to markedly reduce the levels of these cytokines. Moreover, treatment of mice with EPS⁺ *B. breve* has been shown to elicit reduced levels of pro-inflammatory immune cells compared with EPS⁻ strains⁽¹⁶³⁾. A recent study has described the stimulatory effects of a *Lactobacillus*-derived EPS on the release of inflammatory mediators by mouse peritoneal macrophages *in vitro*⁽¹⁵⁸⁾. EPS effectively induced the production of mediators and cytokines by macrophages, especially TNF α , IL6 and IL12. Interestingly, EPS induced higher levels of TNF α and IL6 than of IL10, suggesting a net pro-inflammatory potential. However, its stimulatory effect was significantly lower than that of LPS or whole, killed bacterial cells. Moreover, whole cells were stronger inducers of anti-inflammatory IL10 than EPS alone, suggesting that intact bacteria and EPS may have an opposing effect on macrophage polarisation⁽¹⁵⁸⁾. Similarly, Wu *et al.*⁽¹⁵⁹⁾ investigated the effect of heat-killed *Bifidobacterium longum* and its isolated EPS fraction on the activities of a murine macrophage cell line, including induction of IL10 and TNF α



production. EPS exposure stimulated growth and induced IL10 secretion in macrophages as well as induced lower levels of TNF α secretion. LPS, on the other hand, induced high levels of TNF α secretion and EPS pre-treatment prevented LPS-induced release of TNF α . As both EPS and LPS are surface macromolecules with oligosaccharide moieties, the authors concluded that EPS may act as a LPS blocker. Although EPS may play a role in immune regulation, information about the molecular mechanisms by which EPS interacts with the immune system is scarce⁽¹⁶¹⁾. Defining the common biological properties of EPS has proved to be difficult because of its enormous structural diversity⁽¹⁵⁸⁾.

Some studies have found that bacterial DNA may be partly responsible for the immunomodulatory effect of probiotics. The administration of non-viable, irradiated probiotics (VSL#3) but not heat-killed probiotics has been shown to effectively ameliorate experimental colitis in mice mediated by a TLR9–probiotic DNA interaction⁽¹⁵²⁾. Similarly, *Bifidobacterium* genomic DNA has been shown to induce the secretion of the anti-inflammatory IL10 by human peripheral blood mononuclear cells⁽¹⁵¹⁾.

Immunoactive components of probiotic bacteria may not be limited to cell wall structures and DNA. There have been reports concerning a soluble immunomodulator in bifidobacteria. The immunomodulating activity of *Bifidobacterium adolescentis* increases after disruption of the cells by sonication and the immunopotentiating activity appears in the soluble fraction following centrifugation, indicating the existence of an intracellular soluble immunomodulator⁽¹⁵³⁾. The components of *Bifidobacterium pseudocatenulatum* also have immunomodulatory effects, which appear to be dependent on the method of preparation⁽¹⁶⁴⁾. Compared with heat-treated and untreated cells, sonicated *Bifidobacterium* has been shown to be the most potent inducer of innate immune responses in Peyer's patch cells *in vitro* and *in vivo* (following a single-shot oral administration to mice). However, heat-treated *Bifidobacterium* has been shown to exhibit the greatest immunomodulatory activity following repeated oral administration (for seven consecutive days) in mice. The researchers concluded that the immunomodulatory effect of *Bifidobacterium* is dependent upon the bacterial conformation and condition⁽¹⁶⁴⁾.

Although there is substantial evidence from *in vitro* and animal studies that inactivated probiotics can act as biological response modifiers, there is a relative paucity of information on the effect of dead probiotics *in vivo* in human clinical trials. However, a number of basic human consumption studies have been carried out with Lacteol Fort (*Lactobacillus acidophilus* LB that is heat-killed and freeze-dried in the presence of its fermented culture medium)^(165,166). Lacteol Fort has been shown to improve the clinical symptoms (by decreasing bowel movement, abdominal pain and distension and by improving stool consistency and the feeling of incomplete evacuation) of chronic diarrhoea⁽¹⁶⁵⁾, possibly through a mechanism involving competitive exclusion⁽¹⁶⁷⁾.

In conclusion, while a number of studies have proposed that the viability of probiotics is not essential to exert an immunomodulatory effect, this is not a uniform feature

of all probiotics tested to date, as different *in vitro* studies have also reported that a viable probiotic is essential to exert an immunomodulatory effect^(168,169). In addition, the method of preparation may play a significant role as the immunomodulatory effect may be dependent on the bacterial conformation and condition, as has been described above^(152,164). Further study is essential to elucidate whether inactivated probiotic bacteria or their products are able to exert beneficial effects similar to those exerted by live bacteria *in vivo*. Work on the specific mechanisms is also required to explain what is actually being triggered by the dead agents and whether this is similar to the mechanisms of live agents⁽¹⁵⁶⁾. Biological products based on dead cells might be relatively easy to produce, commercialise and standardise and would have the added advantage of having a much longer shelf life. In addition, use of dead probiotics may be, in some circumstances, safer than using live probiotics. Resolution of this debate might also require participation of relevant stakeholders in re-examining the definition of a probiotic, which is currently restricted to live cells⁽¹²⁵⁾. It must be noted that while the viability of probiotics may not be necessary to exert an immunomodulatory effect, a number of mechanisms mediating the health benefits of probiotics do require viability, such as the metabolism of non-digestible polysaccharides and production of metabolites (e.g. SCFA). Hence, one should be cautious while applying the term 'probiotic' to these dead cell preparations.

Conclusion

The intestinal microbiota is undoubtedly an important factor in determining the health status of the host and has been implicated in both gastrointestinal and extra-intestinal disorders. The studies described in the present review raise the question of whether it is now possible to deduce the composition of a 'healthy' normal microbiota. Indeed, limited accessibility of the different parts of the GIT (including the colonic mucosa) as well as the individual-specific, complex composition of the GIT microbiota makes our understanding of this community somewhat incomplete⁽¹⁷⁰⁾. It is also essential to bear in mind the influence of different dietary patterns on the activity and composition of the microbiota and the potential implications for host health. Although several large international studies are striving to improve our knowledge of the composition of the gut biota, an overwhelming majority of microbes that compose these microbial communities are not yet characterised in detail to any great level⁽⁵⁾. Comparison of data from different studies is difficult as sample processing and analysis methods vary between research groups. Moreover, our apparent inability to culture all members of the microbiota makes it currently impossible to create hypotheses regarding the role of these uncultured microbes in health and disease⁽¹⁷⁰⁾. As we are still unable to completely define the microbiota of a healthy intestinal tract, it is similarly difficult to define the microbiota associated with an intestinal disorder. However, major advances in human metagenomics have now provided a catalogue of 3.3 million non-redundant genes^(5,8). This catalogue will enable the development of gene profiling



approaches that aim to detect associations of bacterial genes and phenotypes. These developments should lead to rapid advances in diagnostic and prognostic tools as well as pave the way to rational approaches to the manipulation of an individual's intestinal microbiota to promote health.

The improvement or maintenance of health through the use of probiotics has been the focus of extensive research. Indeed, the probiotic market has expanded rapidly in recent years and a large variety of probiotic products are available. However, the efficacy of probiotics is strain and dose dependent, and the clinical and methodological differences (strain, dose and formulation) between studies make comparisons difficult. Hence, there is presently strong evidence to support their use only under a few conditions. It is also acknowledged that in most cases, the exact mechanisms of the beneficial effects are not fully understood. Although many studies have provided information on several possible modes of action, it has not been possible to identify definite cause-effect relationships. Stringent requirements imposed by regulatory authorities such as the European Food Safety Authority require more solid scientific evidence to support any health claims associated with probiotic products. Future research requires well-designed, large, randomised, double-blind placebo-controlled clinical trials along with more mechanistic studies on cell and animal models in order to strengthen our evidence base. Indeed, the development of novel *in vitro* models of the human intestinal epithelium (e.g. bioreactors and organoids) will increase our understanding of the molecular mechanisms of host-microbe interactions and pave the way for future ventures aimed at bioengineering human intestine⁽¹⁷¹⁾. Further investigation into the health benefits of ingesting dead organisms *in vivo* is also required. Indeed, the potential health effect of these non-viable bacteria depends on whether the mechanism of the health effect of probiotics is dependent on viability, and hence each probiotic strain should be assessed on a case-by-case basis. Furthermore, as the term 'probiotic' fails to account for the use of dead organisms, it has been suggested that the term 'pharmabiotic' would be more inclusive⁽¹⁷²⁾.

Acknowledgements

The Alimentary Pharmabiotic Centre is a research centre funded by the Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. S. E. P. is supported by the Irish Research Council postgraduate scholarship Enterprise Partnership Scheme (in collaboration with Alimentary Health Limited) and by the Alimentary Pharmabiotic Centre (SFI grant no. 07/CE/B1368). The present review was also supported by the Irish Government's National Development Plan by way of a Department of Agriculture Food and Marine and Health Research Board FHRI award to the ELDERMET project. The above-mentioned funding agencies had no role in the design, analysis or writing of this article.

S. E. P. wrote the manuscript. G. F. F., P. W. O. T., R. P. R. and C. S. critically reviewed the manuscript and contributed to its revision. All authors read, reviewed and approved the final version of the manuscript.

The authors do not have any conflicts of interest.

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