1 Microbiota of the Human Gut[1]

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1.1 Background

The human gastrointestinal (GI) tract has been the subject of intense research over the past decade, since the publication of the first edition of this book. Notably, the Human Microbiome Project in the United States of America (USA) (http://hmpdacc.org) (Turnbaugh *et al*., 2007) and the Metagenomics of the Human Intestinal Tract consortium in Europe (MetaHIT; www.metahit.eu) (Qin *et al*., 2010) have been two major initiatives, but very many other research groups have published their findings. Scientists can get qualitative and quantitative information about all the microbes present in the gut (the gut microbiota) in the context of their habitat, genomes and surrounding environment (the gut microbiome), as well as cataloguing all the metabolites in the gut (metabolomics) and getting an overview of microbial functions in the gut based on analysis of all their genes (metagenomics), the genes' activity (transcriptomics) and proteins present (metaproteomics) (Marchesi *et al*., 2016). Such work has amassed a vast amount of data and helped improve our understanding of microbial communities in the human body. Although the main target of this research has been the human intestinal tract, other body parts, including the skin and the nasal, oral and urogenital tracts, have not been overlooked. Apart from finding an answer to the 'What is there?' question, the main purpose of this research has been to look for associations between any observed changes in the microbiome and the prevalence of certain diseases (Korecka & Arulampalam, 2012). One clear outcome, however, has been the confirmation of the key influence of the human gut microbiota on health, not just of the gut but of the whole body, because of the gut microbiota's influence on different systems in the body (Rooks & Garrett, 2016). In fact, many scientists and medics are now of the opinion that the gut microbiota should be considered equivalent to a body organ (Marchesi *et al*., 2016).

The highly specialised ecosystem that is the human gut microbiota has evolved to achieve a symbiotic homeostatic relationship with the host (Bäckhed *et al*., 2005; Flint *et al*., 2012). The GI tract and its microbiota cannot be really considered as separate

[1] In the book's first edition, this chapter was authored by Dr B. O'Grady and Professor Glenn Gibson of the University of Reading. The current chapter constitutes a major update of that work to reflect the significant advances in this field since 2005.

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entities because together they represent a dynamic biological system that has developed together from birth. The human GI tract is composed of highly adapted regions for mediation of its diverse functions, many of which impact markedly upon host health and welfare. Physiological considerations in each unique region influence the degree and type of colonisation, and initial colonisers also modify the physiological conditions therein. This results in the development of distinct microhabitats along the length of the GI tract, which influence metabolism, protection and immune stimulation (Flint *et al*., 2012; Thomas *et al*., 2014; Honda & Littman, 2016). Such effects are both local and systemic, as the GI tract is connected to the vascular, lymphatic and nervous systems. The ability of the gut to sustain a microbiota that is supportive of health is critical for host health and reduction of disease risk.

1.2 The human GI tract and its microbiota

It has long been thought that colonisation of the GI tract begins immediately after birth (Castanys-Muñoz *et al*., 2016), but although this is certainly when the primary colonisation process occurs, recent studies have reported the detection of micro-organisms in meconium, placenta, umbilical cord and amniotic fluid (Thomas, 2016). Micro-organisms have also been detected in breast milk (Fernández *et al*., 2013).

Microbial colonisation of the neonate mainly occurs during the delivery process. The inoculum may be largely derived either from the mother's vaginal and faecal microbiota (in a conventional birth) or from the environment (in a Caesarean delivery); hence, the micro-organisms that colonise the new-born tract are primarily acquired postnatally. The delivery method is key, as new-borns delivered by Caesarean section are exposed to a different microbiota compared to that found in the vagina. In a recent pilot study, Dominguez-Bello *et al*. (2016) demonstrated that by exposing infants delivered by Caesarean section to maternal vaginal fluids at birth, not only the gut but also the oral and skin bacterial communities of these new-borns were partially altered to become more like those of a naturally delivered infant during the first 30 d of their life. The potential long-term health effects of Caesarean delivery remain unclear, although microbial differences may last for at least one year (Rutayisire *et al*., 2016), and links to health risks such as childhood obesity (Blustein *et al*., 2013) and allergic disease (Brandão *et al*., 2016) have been reported.

Bacterial populations in the gut develop progressively during the first few days of life; facultative anaerobes predominate initially and create a reduced environment that allows for the growth of strict anaerobes (Rodríguez *et al*., 2015). The choice of diet for the new-born is also of importance as the microbiota of breast-fed infants is predominated by bifidobacteria, whereas formula-fed infants have a more complex microbiota that resembles the adult gut, in that *Bacteroides*, clostridia, bifidobacteria, lactobacilli, Gram-positive cocci, coliforms and other groups are all represented in fairly equal proportions (Lozupone *et al*., 2012; Ghoddusi & Tamime, 2014). Breastfeeding promotes a more beneficial microbiota; the presence of certain oligosaccharides in human breast milk, for instance, promotes the growth of beneficial bifidobacteria (Smilowitz *et al*., 2014). During weaning, the microbiota becomes more complex, and the ecosystem is thought to become fairly stable at around two years of age. The prevalence of

Table 1.1 The change in the gut microbiota through life.

Stage of life	Intestinal microbiota profile
Foetus	Usually sterile
Baby	Immediately after birth, there is rapid colonisation of the gut with micro-organisms from the immediate surroundings; the gut microbiota composition is influenced by mode of delivery and type of feeding: • *Breast-fed*: low diversity, dominated by bifidobacteria. • *Formula-fed*: a more diverse microbiota with more Bacteroidetes and fewer bifidobacteria.
Child	The gut microbiota becomes more stable and complex over the first three years (particularly after weaning), so that it becomes much more diverse in its composition and more like that of an adult.
Adults	A diverse composition; dominant phyla are Firmicutes, Bacteroidetes and Actinobacteria.
Old age	The microbiota changes to become less diverse and resilient; there are fewer Firmicutes and bifidobacteria and more Bacteroidetes and Proteobacteria.

bifidobacteria in breast-fed infants is thought to confer protection by improving the colonisation resistance of the gut; among other mechanisms, bifidobacteria exert directly antagonistic activities against gut pathogens. New-borns are susceptible to intestinal infections and atopic diseases as their immune system and GI tract develop. The mode of delivery and subsequent diet, therefore, have important implications, both at birth and later in life, as the initial colonisation process has a strong influence on the development of the GI tract and its microbiota, and in the maturation of the immune system. During the first few years of life and after weaning, the infant microbiota normalises to a composition that remains relatively stable throughout most of adult life (Thomas, 2016). Table 1.1 summarises how the intestinal microbiota develops with age.

In recent years, the development of next-generation sequencing (NGS) techniques has played a major role in revealing that the human body harbours more than 1000 phylotypes, although intestinal bacteria mainly belong to just a few phyla (Tojo *et al*., 2014). Most of this work comes from analysis of faecal samples; these best represent the distal portion of the gut. Due to the difficulties in obtaining samples higher in the gut, it has proved more difficult to get a true picture of the microbial communities in the small and proximal large intestines (Li *et al*., 2015; Marchesi *et al*., 2016).

The GI tract begins with the oral cavity (the mouth, nose and throat), where a complex microbiota exists that comprises viruses, bacteria, archaea and protozoa. Bacterial species cause dental caries and periodontal species, but many bacteria in the oral microbiome remain uncultured (Wade, 2013). Bacteria are found on the posterior and anterior tongue, sub- and supra-gingival plaque, buccal mucosa and vestibular mucosa (Willis *et al*., 1999). These include members of the *Prevotella*, *Porphyromonas*, *Peptostreptococcus*, *Bacteroides*, *Fusobacterium*, *Eubacterium* and *Desulfovibrio* genera. Bacterial numbers drop dramatically to $<10^3$ colony forming units (cfu) mL^{-1} of gastric contents as they encounter the stomach, which provides a highly effective barrier against invading micro-organisms, both pathogenic and benign. Few micro-organisms, with the exception of acid-tolerant lactobacilli, yeasts and notably *Helicobacter pylori*, can survive the harsh, strongly acidic and peristaltic nature of the stomach.

There is a high degree of variability between the stomach, small intestine and colon in terms of numbers and bacterial population types, due predominantly to different transit times, secretions and nutrient availability (Lambert & Hull, 1996; Guilliams, 1999). Micro-organisms themselves are also determinants because they interact with and influence their surroundings to ensure their survival against competitors. This is achieved through many mechanisms, such as increasing aerobic conditions in the gut or producing inhibitory compounds, such as bacteriocins or short-chain fatty acids (which also lower the pH of the gut milieu). Such compounds may also affect the host with positive or negative consequences (Fooks & Gibson, 2002; Fuller & Perdigón, 2003).

The rapid transit time, low pH and presence of bile associated with the small intestine do not provide an environment that encourages the growth of bacteria. The duodenum also has low microbial numbers due to its short transit time and the secretion of intestinal fluids, which create a hostile environment (Sanford, 1992); however, there is a progressive increase in both numbers and species along the jejunum and ileum. The small intestine harbours enterococci, enterobacteria, lactobacilli, *Bacteroides* and clostridia. These rapidly increase in numbers from 10^4–10^6 cfu mL^{-1} in the small intestine to 10^{11}–10^{12} cfu mL^{-1} in the large intestine, as the flow of intestinal chyme slows upon entry into the colon (Salminen *et al*., 1998).

The large gut is favourable for bacterial growth with its slow transit time, ready availability of nutrients and more favourable pH. Several hundred culturable species may be present here, although a significant proportion is not cultivable by conventional methods. The proximal colon is the site of saccharolytic fermentation, due to its high substrate availability (Scott *et al*., 2012; Russell *et al*., 2013; Shanahan, 2013). Organic acids produced from fermentation result in a lower pH (of 5.5–6.0) compared to the more neutral pH found in the distal colon. Transit in the distal colon is slower and nutrient availability is minimised, producing slower growing populations that tend towards more proteolytic fermentations.

An intriguing question about the human microbiota is the relevance of microbial variations in healthy and diseased individuals, and whether microbial mapping could help predict specific conditions (Knights *et al*., 2014). Despite the diverse range of micro-organisms found in the human digestive tract, it has been suggested that just five or six genera and two phyla shape the mainstream biomass. Numerically dominant genera include *Bacteroides*, *Bifidobacterium* and *Eubacterium* and, to a lesser extent, although still important, *Clostridium*, *Enterobacteriaceae* and *Streptococcus* (Gibson & Roberfroid, 1995; Salminen *et al*., 1998). Five bacterial phyla represent the bulk of the bacteria in the gut, with the two major phyla being the Gram-positive Firmicutes and the Gram-negative Bacteroidetes (LePage *et al*., 2013), which have relatively similar proportions in different individuals (Jeffery *et al*., 2012). In 2011, three different profiles for the human gut microbiota were proposed, termed ‘enterotypes’, that were dominated by *Bacteroides*, *Prevotella* or *Ruminococcus* (Arumugam *et al*., 2011). The situation, however, may be more complex than this, and further research is also needed to elucidate the health implications of such enterotypes (Gibson *et al*., 2016).

Table 1.2 illustrates the representation of the microbiota of the GI tract, highlighting some of the common bacteria and their abundance in different parts of the human digestive system. Yeasts, including the opportunistic pathogen *Candida albicans*, are also

Table 1.2 Representative bacteria in the gastrointestinal (GI) tract.

Bacterial family or genus	GI tract region	Microbial count (colony forming units (cfu) mL^{-1}	Function of the GI tract region
Lactobacillus *Streptococcus* *Helicobacter* *Peptostreptococcus*	Stomach	$1–10^2$	• Hydrochloric acid secretion • Macromolecule digestion • pH 2
Streptococcus *Lactobacillus*	Duodenum Jejunum Ileum	$10^1–10^3$ $10^3–10^4$ $10^7–10^9$	• Main digestion • Absorption of monosaccharides, amino acids, fatty acids and water • pH 4–5
Bacteroides *Clostridium* *Streptococcus* Actinomycineae	Caecum	NR[1]	• Absorption of fluids and salts • Mixing of the lumen contents with mucus • pH 5.7
Bacteroides *Clostridium* *Bifidobacterium* Enterobacteriaceae *Eubacterium*	Colon	$10^{11}–10^{12}$	• Microbial production of secondary bile acids and vitamin B_{12} • Water absorption • pH 7
NR	Rectum	NR	• Storage of faeces before evacuation • pH 6.7

NR = Not reported.
Adapted from Korecka and Arulampalam (2012).

present in the gut microbiota, although in healthy individuals its counts do not exceed 10^4 cfu g^{-1} in faeces (Bernhardt *et al*., 1995; Bernhardt & Knoke, 1997). The vast majority (>90%) of the total cells in the body are present as bacteria in the colon. It is thought that over 60% of the faecal mass exists as prokaryotic cells. As well as the different microhabitats along the length of the GI tract, there are other microhabitats, such as the surface of the gut epithelia, the gut lumen, the colonic mucus layers and the ileum/caecum and colon (Donaldson *et al*., 2016).

The classification of the microbiota as autochthonous or allochthonous complements the distinction between these different habitats of the GI tract (Savage *et al*., 1968). Autochthonous micro-organisms are indigenous and colonise the GI tract, whereas allochthonous micro-organisms are transient and will predictably be found in the lumen. The slow transit time of the large intestine allows multiplication of the luminal microbiota; allochthonous micro-organisms exert equally important effects on the GI tract as their autochthonous counterparts.

1.3 Functions of the GI microbiota

The GI tract along with its microbiota comprise one of the most metabolically active organs in the human body. The intestinal microbiota is involved in the fermentation of endogenous and exogenous microbial growth substrates. The metabolic end products of carbohydrate fermentation are benign or even advantageous to human health (Macfarlane

& Gibson, 1994; Flint *et al*., 2012; Rooks *et al*., 2016). Major substrates available for the colonic fermentation are starches that, for various reasons, are resistant to the action of pancreatic amylases but can be degraded by bacterial enzymes, as well as dietary fibres, such as pectins and xylans. Other carbohydrate sources available for fermentation in lower concentrations include oligosaccharides and a variety of sugars and non-absorbable sugar alcohols. Saccharolysis results in the production of short-chain fatty acids (SCFAs), such as butyrate, acetate, propionate and lactate that contribute towards the energy metabolism of the large intestinal mucosa and colonic cell growth; they can also be metabolised by host tissues, such as the liver, muscle and brain. The production of SCFAs concomitantly results in a lower pH that can protect against invading micro-organisms and also reduces the transformation of primary bile acids into secondary pro-carcinogenic bile acids (Cummings & Macfarlane, 1997; Marchesi *et al*., 2016). This is one of the mechanisms utilised by beneficial bacteria in the gut that results in protection for the host.

Proteins and amino acids can be effective growth substrates for colonic bacteria, whilst bacterial secretions, lysis products, sloughed epithelial cells and mucins may also make a contribution. However, diet provides, by far, the predominant source of nutrients, with around 70–100 g d^{-1} of dietary residues available for the colonic microbiota. These materials are degraded by a wide range of bacterial polysaccharidases, glycosidases, proteases and amino-peptidases to smaller oligomers and their component sugars and amino acids (Macfarlane & Gibson, 1994).

The gut profile of each adult represents a population of microbes that has evolved since birth and that can best cope with the physiological and microbiological pressure encountered within this ecosystem. This stability provides resistance for the host, also known as the 'barrier effect', against invading micro-organisms, both pathogenic and benign. The indigenous gut microbiota is better adapted to compete for nutrients and attachment sites than any incoming micro-organism, which it may also inhibit through the production of compounds (Alderbeth *et al*., 2000). The role of the intestinal microbiota in challenging invading micro-organisms and preventing disease through competitive exclusion is best demonstrated by the studies showing that germ-free animals are more susceptible to infection (Baba *et al*., 1991). This demonstrates the individual role of beneficial micro-organisms in preventing infection through colonisation resistance.

Another important function of the gut microbiota is the production of vitamins B and K; this is best demonstrated by studies where germ-free animals required a 30% increase in their diet to maintain their body weight, and supplementation with vitamins B and K as compared to animals with a microbiota (Hooper *et al*., 2002).

The ability of the gut microbiota, however, to utilise biologically available compounds can have negative outcomes. *Helicobacter pylori* can affect the absorption of vitamin C and important micronutrients for host health (Annibale *et al*., 2002). Moreover, the fermentation of proteins and amino acids in the distal colon can lead to the production of toxic substances such as ammonia, phenols and amines that are undesirable for host health (Mykkanen *et al*., 1998; Kim *et al*., 2013). This highlights the importance of ensuring a balance of beneficial bacteria to prevent the multiplication of pathogens or bacteria whose growth and metabolism may increase disease risk.

The GI tract is in more contact with the external environment than our skin, which exposes ~2 m^2, whereas the GI tract exposes a surface area of ~200 m^2 (Guilliams, 1999). The microbiota of the GI tract is therefore heavily involved in gut maturation. As mentioned in this chapter, exposure to the intestinal microbiota after birth plays a critical role in stimulating local and systemic responses and supporting the maturation of the immune system. The intestinal microbiota also provides a source for non-inflammatory immune stimulation, throughout life, by stimulating the production of secretory IgA, which neutralises foreign bacteria and viruses (Moreau, 2000; Mathias *et al*., 2014). The immune system–microbiota alliance provides a dynamic environment by defending the host from pathogens as well as maintaining a balanced and controlled tolerance to harmless antigens. Many factors can play a role in destabilising this coalition and disturbing this symbiotic relationship, including changes in diet and overuse of antibiotics, which in turn could allow the proliferation of a microbiota lacking in diversity or the resilience and tolerance needed for a well-functioning immune system. The rise in autoimmune diseases and inflammatory disorders has been suggested to be partly the result of this troubled reciprocal relationship. Overall, the ability of the GI tract to perform its functions of nutrient uptake in conjunction with the exclusion of foreign antigens or micro-organisms is a complex and difficult process. The interplay between the host immune response and the GI microbiota is critical to health; loss of tolerance may become clinically manifest through disorders, such as inflammatory bowel disease (IBD) (Malloy & Powrie, 2011).

The gut microbiota and host health has found a new clinical frontier in recent years, the so-called gut–brain axis (El Aidy *et al*., 2015), which is described as a two-way communication between the central and the enteric nervous systems, in which the emotional, intuitive, decision-making and cognitive centres of the brain are linked with peripheral intestinal functions (Mayer, 2011). This bidirectional interaction is believed to include signal exchange between gut microbiota and the brain through neural, endocrine, immune and humoral links (Carabotti *et al*., 2015; Kountouras *et al*., 2015). To provide evidence of these interactions, studies on germ-free animal models, probiotics, antibiotics and infection have been carried out. At a clinical level, studies have focused on central nervous disorders such as autism, anxiety-depressive behaviours and GI disorders, such as (typically) irritable bowel syndrome. It is hoped that such investigations lead to new therapeutic strategies (Distrutti *et al*., 2016).

1.4 Influences on the GI tract and its microbiota

The profile of the intestinal microbiota that develops in each individual is a result of their host genetics (as shown in twin studies in the UK) (Goodrich *et al*., 2014), environmental factors and microbiological influences. These factors result in a stable community of micro-organisms that is more unique than an individual's own fingerprint; even homozygotic twins develop distinct microbial profiles (Zoetendal *et al*., 2001). Notwithstanding this, the overall metabolism of a healthy gut ecosystem varies little from one individual to another, as evinced by the ratios of major metabolic end products. Modern living presents numerous challenges to the human GI tract, particularly in

the developed world, with often stressful lifestyles and unhealthy intake of processed foods. Antibiotics and other medications, however, can cause immediate serious disruption of the gut microbiota, and the resulting dysbiosis may be long term (Jernberg *et al*., 2010; Francino, 2015). Disturbances of the microbiota can have serious implications, and this fragility merits careful consideration of the external influences on the GI tract and how they may disrupt host health (O'Sullivan *et al*., 2013). The numerous factors which act upon the intestinal microbiota are briefly outlined in Table 1.3; some of the more relevant influences are discussed here.

The influence of diet on the neonatal intestinal microbiota has already been outlined (do Rosario *et al*., 2016; Ojeda *et al*., 2016). The GI tract of healthy humans remains relatively stable throughout life apart from later life, when a significant decrease of beneficial bifidobacteria and loss of microbial diversity have been reported. Such changes have also been linked to indications of increased risk of disease and frailty (van Tongeren *et al*., 2005; Claessen *et al*., 2012; Jackson *et al*., 2016). Diet is an effective and rapid modulator of the microbial composition and metabolic activity of the human gut, which in turn can impact health (Claesson *et al*., 2012; Conlon & Bird, 2015) with temporary and/or lasting effects. For example, the ELDERMET study in Ireland has shown clear differences between the core microbiota in older people compared to younger ones. Furthermore, clear differences were observed in the gut microbiota that correlated to these older persons' place of residence: long-term residential care, rehabilitation hospital care for less than six months, attending hospital outpatients or living in the community (Claessen *et al*., 2012). The profile of the microbiota of those living at home was the one most similar to that of healthy younger adults, whereas the gut microbiota of the older people living in long-term care was significantly different and much less diverse. These microbiota differences correlated with the different diets eaten at home or in residential care; the latter had a much lower intake of fruit, vegetables and fibre, and a higher intake of fatty, starchy and sugary foods. Whilst long-term diet clearly influences the composition of gut microbiota, even short-term dietary modifications lead to significant and relatively swift changes in the composition of the microbiota, but

Table 1.3 Influences on the composition of the gastrointestinal microbiota.

- Type of feeding
- Amount, chemical composition and availability of growth substrate
- 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

Adapted from Fooks *et al*. (1999).

these would not be expected to cause a lasting shift in microbiota composition or affect the core profile. Data indicate that such changes may be at genus and species level, but not at phylum level (Wu *et al*., 2011).

Type of dietary intake has consequences in the colon as carbohydrate fermentations usually result in benign end products (Wong *et al*., 2006; do Rosario *et al*., 2016). However, when carbohydrate levels become diminished, proteolytic fermentation in more distal regions produces toxic compounds that can predispose to diseases such as colorectal cancer or ulcerative colitis (Nyangale *et al*., 2012); thus, protein-based diets such as the Atkins diet could potentially have serious long-term repercussions for gut health (Russell *et al*., 2011). High intakes of processed food and other dietary aspects will reduce levels of fibre in the diet, which is of concern as dietary fibre influences stool volume, colon motility, water absorption and faecal transit time (Dhingra *et al*., 2012).

Chronic illness, immune suppression and the use of broad-spectrum antibiotics can severely compromise the crucial balance between beneficial and harmful micro-organisms in the gut microbiota. The loss of any beneficial genera sensitive to antibiotic therapy, such as lactobacilli and bifidobacteria, has implications for GI health, as opportunistic pathogens can overgrow the gut, and the host will have increased risk for iatrogenic disease. For example, the serious concerns about the risks of antibiotic-associated diarrhoea, including that caused by *Clostridium difficile*, are well documented (Burke & Lamont, 2014; Elseviers *et al*., 2015).

The increase in antibiotic resistance, the lack of progress in developing new antibiotics, concerns over (possibly long-term) adverse effects associated with antibiotic use (such as increased risk of obesity) (Reid, 2006; Langdon *et al*., 2016; Ouwehand *et al*., 2016) plus consumer interest in dietary supplements to maintain GI health have fuelled scientific research into alternative strategies. The potential for preventing dysbiosis, increasing the resilience of the gut microbiota or otherwise fortifying the GI tract through modulation of the intestinal microbiota has been widely explored. The principle of using harmless bacteria to prevent disease dates back to the suggestion of Metchnikoff at the turn of the twentieth century that ingested bacteria could promote longevity and well-being (Metchnikoff, 1907; see Chapter 2 for details). Micro-organisms associated with health benefits *in vivo* include many members of the *Lactobacillus* and *Bifidobacterium* genera, although *Escherichia coli*, streptococci, enterococci, lactococci, bacilli and yeasts, such as *Saccharomyces cerevisiae* var. *boulardii*, have also been used (Table 1.4). Such strains have been researched for their probiotic potential, and many strains (including those marketed commercially) are the focus of intense research (see Chapter 8 for further details).

1.5 Conclusions

A number of disease states have been linked to dysbiosis and/or low diversity of the gut microbiota, suggesting that its manipulation at any stage of life but particularly in infancy could have beneficial consequences in reducing the risk of both short-term and long-term disease (Thomas *et al*., 2014; Carding *et al*., 2015; Prosberg *et al*., 2016). Differences in the ratio of Firmicutes to Bacteroidetes have also been observed between

Table 1.4 Examples of microbial species that contain probiotic strains.

Microbial genus or group	Species
Bifidobacterium	*Bifidobacterium bifidum*
	Bifidobacterium longum subsp. *longum*
	Bifidobacterium breve
	Bifidobacterium adolescentis
	Bifidobacterium longum subsp. *infantis*
Enterococcus	*Enterococcus faecalis*
	Enterococcus faecium
Lactococcus	*Lactococcus lactis* subsp. *lactis*
Lactobacillus	*Lactobacillus acidophilus*
	Lactobacillus rhamnosus
	Lactobacillus reuteri
	Lactobacillus casei
	Lactobacillus gasseri
	Lactobacillus plantarum
Yeast	*Saccharomyces cerevisiae* var. *boulardii*

individuals and patient groups. Other examples include IBD, where low counts of *Faecalibacterium prausnitzii* have been associated with increased risk of ulcerative colitis (Sokol *et al*., 2009), and several species have been implicated in colorectal cancer, including *Streptococcus gallolyticus*, *Enterococcus faecalis* and *Bacteroides fragilis* (Wu *et al*., 2009; Boleij & Tjalsma, 2013; Wang *et al*., 2015).

A key question in gut microbiota research, however, is whether such microbial changes are the *cause* of the disease or are the *result* of disease (Zhang, 2013). One tactic to explore this 'correlation/causality' microbial conundrum is to conduct clinical trials in patients or people at risk of disease, investigating the health effects of modulating the microbiota. Faecal microbiota transplantation, for example, has shown strong efficacy for treatment of *C. difficile* infection (Borody *et al*., 2015). Probiotics work through multiple mechanisms of activity, including the modulation of the gut microbiota, and evidence of probiotic benefit for a broad range of disorders has accumulated. This is discussed further in Chapter 8.

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