

Intestinal microbiota in human health and disease: the impact of probiotics

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Received: 4 February 2011 / Accepted: 20 April 2011 / Published online: 27 May 2011
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Abstract The complex communities of microorganisms that colonise the human gastrointestinal tract play an important role in human health. The development of culture-independent molecular techniques has provided new insights in the composition and diversity of the intestinal microbiota. Here, we summarise the present state of the art on the intestinal microbiota with specific attention for the application of high-throughput functional microbiomic approaches to determine the contribution of the intestinal microbiota to human health. Moreover, we review the association between dysbiosis of the microbiota and both intestinal and extra-intestinal diseases. Finally, we discuss the potential of probiotic microorganism to modulate the intestinal microbiota and thereby contribute to health and well-being. The effects of probiotic consumption on the intestinal microbiota are addressed, as well as the development of tailor-made probiotics designed for specific aberrations that are associated with microbial dysbiosis.

Keywords Diversity · Dysbiosis · Host-microbe interactions · Intestinal microbiota · Probiotics

Introduction

It is known for over three decades that the human body contains tenfold more microbial cells (10^{14}) than human cells (Savage 1977). These microorganisms colonise practically every surface of the human body that is exposed to the external environment, including the skin, oral cavity, respiratory, urogenital and gastrointestinal tract. Of these body sites, the gastrointestinal (GI) tract is by far the most densely colonised organ. The complex community of microorganisms residing in or passing through the GI tract is referred to as the intestinal microbiota.

The intestinal microbiota plays a role in metabolic, nutritional, physiological and immunological processes in the human body. It exerts important metabolic activities by extracting energy from otherwise indigestible dietary polysaccharides such as resistant starch and dietary fibres. These metabolic activities also lead to the production of important nutrients, such as short-chain fatty acids (SCFA), vitamins (e.g. vitamin K, vitamin B12 and folic acid) and amino acids, which humans are unable to produce themselves (Hamer et al. 2008; Wong et al. 2006). In addition, the intestinal microbiota participates in the defence against pathogens by mechanisms such as colonisation resistance and production of antimicrobial compounds. Furthermore, the intestinal microbiota is involved in the development, maturation and maintenance of the GI sensory and motoric functions, the intestinal barrier and the mucosal immune system. These are just a few examples of the functional contributions of the intestinal microbiota to human health, a subject that is regularly reviewed (Barbara et al. 2005;

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Cerf–Bensussan and Gaboriau–Routhiau 2010; O’Hara and Shanahan 2006; Sekirov et al. 2010; Zoetendal et al. 2008).

In recent years, a sharp increase is seen in the number of publications addressing the intestinal microbiota. They have provided various lines of evidence supporting a close link between the intestinal microbiota and human health. This review aims to summarise the current knowledge on the composition and diversity of the intestinal microbiota. In addition, it is discussed how new molecular approaches have provided novel insights towards the phylogenetic and functional characterisation of the intestinal microbiota. Furthermore, recent insights on the link between the intestinal microbiota and human health are provided. Finally, an overview is presented of ways to modulate the intestinal microbiota with specific attention for the use of probiotics, defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2002).

Microbial diversity in the GI tract

The GI tract is a complex and dynamic ecosystem containing a diverse collection of microorganisms. These microorganisms are either resident members of the intestinal microbiota or transient passengers introduced from the environment, for example by the regularly influx of microorganisms by the intake of food.

Compositional diversity of the intestinal microbiota

The intestinal microbiota can be described in richness (‘who is present’) and evenness (‘with how many are they present’) that together form the ecological terms of diversity. If applied at the species-level, richness describes the number of species present in a specific ecosystem, not taking into account their relative abundance. This contrasts with evenness, which represents the relative abundance of

each species in a specific ecosystem. These definitions are used to describe the microbial diversity in the GI tract.

Up till recently, conventional culture-based methods were used to assess the intestinal microbial diversity. Over 400 bacterial species have been successfully isolated, cultured and characterised from the human GI tract (Rajilić-Stojanović et al. 2007). However, these culture-based methods have proven to be inadequate in determining the true microbial diversity of the intestinal microbiota since a large fraction of the microbiota remains uncultivated. For a more accurate analysis of the compositional diversity of the intestinal microbiota culture-independent approaches have been developed and it has been revealed that the human intestinal microbiota is an even more complex ecosystem than previously expected. Most of these techniques target the highly conserved 16S ribosomal RNA (rRNA) gene sequences of bacterial and archaeal microorganisms. Molecular techniques that are used to study the diversity of the intestinal microbiota include quantitative polymerase chain reaction (qPCR), temperature or denaturing gradient gel electrophoresis (TGGE or DGGE), terminal-restriction fragment length polymorphism (T-RFLP) and fluorescent in situ hybridisation (FISH). The latest developments in high-throughput technologies, such as next generation sequencing and phylogenetic micro-arrays, now allow more in-depth analysis of the complete phylogenetic diversity of the intestinal microbiota (Van den Bogert et al. 2011; Zoetendal et al. 2008). Moving beyond the analysis of the variation in the sequence of a single marker gene, it is currently also possible to characterise the complete genetic material obtained from environmental samples such as the GI tract. With the aid of large-scale sequencing approaches these so called metagenomes can be studied and so far several metagenomic inventories of the intestinal microbiota have been reported (Table 1).

Since the first application of culture-independent methods to determine diversity, it has been shown that the composition of the intestinal microbiota varies substantially amongst individuals (Zoetendal et al. 1998). At least

Table 1 Overview of metagenomic studies of the human intestinal microbiota

Nationality of individuals	Number of individuals	Sequencing technology	Total length of sequences obtained (Gb)	References
American	2	Sanger	0.2	Gill et al. (2006)
Japanese	13	Sanger	0.727	Kurokawa et al. (2007)
American	18	454 FLX Titanium	2.14	Turnbaugh et al. (2009)
European (Danish or Spanish)	124	Solexa (Illumina)	576.7	MetaHIT Qin et al. (2010)
European	20	Sanger	2.6	Genescope
French	49	Solid	200	INRA

part of this diversity can be attributed to genetic differences amongst hosts. A positive relation between similarity in dominant faecal microbial communities and genetic relatedness of the hosts has been observed (Stewart et al. 2005; Turnbaugh et al. 2009; Zoetendal et al. 2001). It is estimated that more than 1,000 species-level phylotypes can be found in the GI tract of the total human population (Qin et al. 2010; Rajilić-Stojanović et al. 2007). However, the phylogenetic diversity in one individual is much lower, since the intestinal microbiota of each individual only consists of approximately 160 different bacterial species (Qin et al. 2010). This estimation is based on metagenomic analysis using the number of non-redundant genes contained by an average-sized genome. Despite the high species richness and inter-individual variability of the intestinal microbiota, a limited number of bacterial phylotypes is more prevalent amongst individuals and might therefore represent a shared phylogenetic core (Qin et al. 2010; Tap et al. 2009). However, the estimation of the size of the phylogenetic core is dependent on the minimal relative abundance of a given phylotype that can be detected by the molecular approaches deployed. Recent analysis of metagenomic data indicated that there is a high variability in relative abundance (evenness) of core phylotypes amongst individuals (12- to 2,200-fold difference) (Qin et al. 2010). Altogether, these results demonstrate that an accurate estimation of the size of the phylogenetic core is still difficult to make as this is highly dependent on the depth of the analysis.

The vast majority of all microbial cells in the human GI tract are bacteria. At the phylum-level, both culture-dependent and independent studies have demonstrated that the majority of the intestinal bacteria belong to two phyla, the *Bacteroidetes* and the *Firmicutes* (Mariat et al. 2009). The phylum *Bacteroidetes* consists of three classes, of which the class *Bacteroidetes*, containing the well-known genera *Bacteroides* and *Prevotella*, is probably the most well studied. The *Firmicutes* is currently the largest bacterial phylum, which contains more than 200 genera. The majority of the *Firmicutes* detected in the GI tract fall primarily into two main groups, the *Clostridium coccoides* group (also known as *Clostridium* cluster XIVa) and the *Clostridium leptum* group (also referred to as *Clostridium* cluster IV) (Collins et al. 1994; Mariat et al. 2009). Both groups contain members of the genera *Clostridium*, *Eubacterium* and *Ruminococcus* that are taxonomically polyphyletic. In addition to the two phyla *Bacteroidetes* and *Firmicutes*, also members of other phyla, such as *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Spirochaetes*, *Verrucomicrobia* and *Lentisphaerae*, have been detected (Rajilić-Stojanović et al. 2007; Zoetendal et al. 2008).

Although bacteria dominate the GI tract ecosystem, species from the archaeal domain can also be found in the

GI tract, with the methanogens, *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* being by far the most dominant archaeal groups (Gill et al. 2006; Mihajlovski et al. 2008). While it was previously assumed that these methanogens were only present in a minor fraction of healthy subjects, application of new DNA isolation methods has led to the observation that they are in fact highly prevalent (Dridi et al. 2009; Salonen et al. 2010b). In addition to bacteria and archaea, eukaryotic microorganisms can also be members of the intestinal microbiota. Culture-independent analysis of the fungal diversity in the GI tract has demonstrated that the majority of the phylotypes belonged to the two fungal phyla *Ascomycota* (which includes the genera *Candida* and *Saccharomyces*) and *Basidiomycota* (Ott et al. 2008; Scanlan and Marchesi 2008).

Microbial diversity along the GI tract

Host physiology and intestinal microbiota are intimately connected. This is evident from the fact that each distinct anatomical region along the GI tract is characterised by its own physicochemical conditions, and that these changing conditions exert a selective pressure on the microbiota. The physicochemical conditions that influence the composition of the intestinal microbiota include intestinal motility, pH, redox potential, nutrient supplies, host secretions (e.g. hydrochloric acid, digestive enzymes, bile and mucus), and the presence of an intact ileocaecal valve (Booijink et al. 2007). Thus, the GI tract harbours many distinct niches, each containing a different microbial ecosystem that varies according to the location within the GI tract. This is already demonstrated by the fact that the microbial density increases along the GI tract. Per gram of intestinal content, the microbial density increases from 10^1 – 10^4 microbial cells in the stomach and duodenum, 10^4 – 10^8 cells in the jejunum and ileum, to 10^{10} – 10^{12} cells in the colon and faeces (Booijink et al. 2007; Dethlefsen et al. 2006). Despite the fact that it is well known that the intestinal microbiota is not homogeneously distributed within the GI tract, it is still largely unknown how the diversity varies in the different niches along the GI tract ('who is present where').

By far, the most detailed knowledge is available with respect to the microbial composition of faeces. This is mainly because faecal material can be collected non-invasively and contains a large biomass of microbial cells. However, as it is increasingly acknowledged that the composition of microbiota differs significantly in the different niches, more efforts are undertaken to determine the spatio-temporal dynamics of the microbial diversity along the whole GI tract (Zoetendal et al. 2002). The large intestine has a rather uniform composition of luminal

intestinal microorganisms, and faecal material seems to represent the colonic microbiota composition best (Eckburg et al. 2005). In contrast, there is only limited insight in the composition of the microbiota that resides in the small intestine. Especially the lower part of the small intestine, the ileum, has received minimal attention, mainly due to sampling difficulties caused by the inaccessibility of this region (Booijink et al. 2007). The composition of the small intestinal microbiota is largely influenced by a combination of gastric acid, bile and pancreatic secretions that enter the GI tract in the duodenum, and which together create a harsh environment for most microorganisms (Booijink et al. 2007). Hence, compared with other regions, few microorganisms are able to inhabit the upper part of the GI tract. In addition, the antegrade peristaltic movements as part of the migrating motor complex (MMC) ensure a relatively short passage time through the small intestine (3–5 h) by pushing the microbiota towards the large intestine, thus leaving limited time for microorganisms to replicate and increase in numbers (Booijink et al. 2007). The short passage time allows transitioning bacteria to retain viability. Furthermore, cellular enzymes such as glutamate decarboxylase and bile resistance systems offer protection against the low pH and bile salts encountered in this upper part of the GI tract, respectively (Audia et al. 2001; Merritt and Donaldson 2009).

The small intestine is the part of the GI tract where most of the host enzymatic digestion of the food occurs. The products of these digestive activities are absorbed in more distal parts of the small intestine, the jejunum and especially the ileum. The conditions in the ileum are more favourable for microbial growth compared with the proximal part of the small intestine, as for example the pH is less acidic and bile acids are reabsorbed. Therefore, the number of microorganisms in the ileum can be higher compared with the duodenum (Booijink et al. 2007).

Most of the knowledge about the small intestinal microbiota has been derived from studies with ileal biopsies collected during surgical intervention (Ahmed et al. 2007; Baumgart et al. 2007; Wang et al. 2003, 2005; Willing et al. 2009) or from samples obtained from elderly individuals at autopsy (Hayashi et al. 2005). In addition, ileal effluent from ileostomy patients has been used to study the diversity of the luminal microbiota of the human ileum (Booijink et al. 2010; Hartman et al. 2009). It was shown that the composition of the microbiota in ileostomy effluent clearly differs from that of the faecal microbiota. Compared with faecal microbiota, ileostomy effluent microbiota is less diverse and less stable, since large fluctuations in ileal microbiota profiles per individual were observed over time (Booijink et al. 2010). One of the main findings of this study by Booijink and colleagues was that ileostomy effluent showed a higher relative abundance of species

within the orders *Lactobacillales* and *Clostridiales*, especially *Veillonella*- and *Streptococcus*-related phylotypes (Booijink et al. 2010). In addition, species belonging to *Clostridium* cluster I were detected in high levels, in contrast with the reduced levels of species belonging to the *Bacteroidetes* and *Clostridium* clusters III, IV and XIVa. More recently, it was demonstrated that the microbiota composition of ileostomy effluent, which is characterised by an abundance in *Streptococcus* and *Veillonella* species, is more similar to the proximal small intestinal microbiota and clearly differs from that of the ileum (Zoetendal et al. 2011).

In addition to the variation in microbial composition along the GI tract, the microbiota present in the intestinal lumen also differs significantly from that attached to and imbedded in the intestinal mucus layer. Since mucosa-associated microorganisms live in close contact with host cells, it is likely they execute different functions within the GI ecosystem compared with luminal microorganisms. Several studies have reported a significant difference in dominant microbial community composition between colonic biopsies and faecal samples in humans (Eckburg et al. 2005; Lepage et al. 2005; Zoetendal et al. 2002). It should be kept in mind, however, that in these studies colonic biopsies were obtained from humans undergoing standard colonoscopy, which in general is preceded by a laxative preparation in order to clean the GI tract. The influence of this procedure on the luminal and mucosa-associated microbiota is still largely unknown (Mai et al. 2006).

Animal models could provide a means to study both the microbial composition along the GI tract as well as the difference in luminal and mucosa-associated microbiota, without the need for physiological alterations during sampling. It has been demonstrated in rodents that intestinal microorganisms are able to survive and even proliferate in the outer loose mucus layer since the glycans present in this layer are accessible as energy source for these microorganisms (Kim and Ho 2010; Johansson et al. 2010). In contrast, the inner stratified firmly attached mucus layer probably prevents the intestinal bacteria from coming in contact with the colonic epithelial cells (Johansson et al. 2008). The organisation of the mucus layers varies amongst the different parts of the GI tract, as it has been observed that the mucus layers in the stomach and the colon are well defined, in contrast to the small intestine where the mucus is less evenly distributed (Atuma et al. 2001; Johansson et al. 2010). Most likely, such differences in mucus layer organisation will be associated with variation in the mucosa-associated microbiota along the GI tract. A recent study in mice has shown that the dominant microbiota composition of proximal colonic mucosa-associated and faecal microbiota are very similar to each other, but differ

both significantly from distal colonic mucosa-associated samples (Wang et al. 2010). In addition, the study demonstrated that the region-specific mucosa-associated microbiota determines the region-specific expression of host genes, in this case of genes encoding Toll-like receptors (TLRs).

Due to the application of culture-independent molecular approaches, our knowledge of the intestinal microbiota has been advanced significantly (Zoetendal et al. 2006). Yet, a complete description of the microbial diversity along the human GI tract cannot be given at this moment. Future research should include more samples from the various distinct niches along the GI tract, which nowadays can be collected using minimally invasive methods and which can be deeply analysed using high-throughput technologies.

Functional diversity of the intestinal microbiota

Recently, the collective genome of the human intestinal microbiota (the human intestinal microbiome) was estimated to contain 3.3 million microbial genes, which is ~150 times more genes than the human genome (Qin et al. 2010). The presence of this wide array of genes in addition to our own genome, suggests that a profound influence of intestinal microorganisms on the human body can be expected. This means that meaningful information related to human health does not only originate from insights in the compositional diversity ('who is present', 'with how many are they present' and 'who is present where'), but can also be derived from knowledge on the function of the microbiota ('what are they doing'). The extent to which the intestinal microbiota is able to expand the metabolic, nutritional, physiological and immunological functions the host is able to perform, is still largely unknown. To address this question, metagenomic studies can provide information on the diversity of the genes encoded by the intestinal microbiota. Recently, it was calculated that almost 40% of the microbial genes present in each human individual were shared with at least half of the human individuals in the studied cohort. These data provide evidence for the existence of a functional core (core microbiome) (Qin et al. 2010). Since functional redundancy within members of the intestinal microbiota exists, there is the possibility that the phylogenetic core does not fully correspond to the functional core (Zoetendal et al. 2008). The functional core may contain shared metabolic functions (e.g. degradation of sugar monomers, production of vitamins or butyrate formation) as well as sequential pathways which would, respectively, restrict or expand functional diversity irrespective of phylogenetic diversity.

A main focus of current research is to understand the functional contribution of the human intestinal microbiota to the host. Function-driven metagenomics is a first step in assessing the functional capacity of the intestinal microbiota. A prediction of the functional capacity can originate

from the metagenome by comparing the assembled sequences to reference databases, such as the COG (clusters of orthologous groups) and KEGG (Kyoto encyclopedia of genes and genomes) databases. Moreover, function-driven metagenomics can be applied to assign a function to predicted gene products and can even contribute to gene discovery (Tasse et al. 2010; Cowan et al. 2005). The first metagenomic studies have demonstrated that, compared with the human genome, the human intestinal microbiome is highly enriched for COG and KEGG categories involved in metabolism (Gill et al. 2006; Kurokawa et al. 2007; Turnbaugh et al. 2009). Pathways involved in metabolism of energy, amino acids, nucleotides, carbohydrates, cofactors and vitamins, terpenoids and polyketides, and the biosynthesis of secondary metabolites are highly represented in the human microbiome. These pathways not only allow the microbes to generate energy, to grow and proliferate, but also to influence the host. Some of the metabolites are being taken away from the host while other ones are provided (e.g. SCFA, vitamins, gases). Overall, the (metabolic) interaction between microbes and host is beneficial for both parties. Future studies should provide data to further establish and detail the functional contribution of the intestinal microbiota to the metabolic capacity of the host.

Metagenomic studies provide only insight in the genetic potential of the intestinal microbiota and do not demonstrate its true functional contribution to the maintenance of health and well-being (Zoetendal et al. 2008). In order to obtain insights in the *in situ* expression of genes encoded by the intestinal microbiome, other functional microbiomic approaches, such as metatranscriptomics, metaproteomics and metabolomics are required. A recent example of a metatranscriptomic approach to study the intestinal microbiota is provided by the study performed by Booijink et al. (2010). These authors were able to demonstrate that the gene expression of the human faecal microbiota is subject-specific and enriched for genes involved in (carbohydrate) metabolism. Gosalbes and colleagues also applied a metatranscriptomic approach to study the functionality of the faecal microbiota of healthy volunteers (Gosalbes et al. 2011). Remarkably, more rRNA genes were observed than protein-encoding genes. Analysis of the latter showed a uniform functional pattern in carbohydrate metabolism, energy production and synthesis of cellular components as well as regulatory elements (small RNAs). More specific information has been derived from the metatranscriptomic analysis of bifidobacteria in early life that revealed marked differences between breast-fed and formula-fed infants. Moreover, the specific expression of genes involved in the degradation of human-derived sugars and vitamins such as folic acid biosynthesis testify for the health impacting function of intestinal bifidobacteria (Klaassens et al. 2009). Furthermore, metaproteomics

approaches have been applied to investigate faecal samples obtained from human infants (Klaassens et al. 2007) and adults (Rooijers et al. 2011; Verberkmoes et al. 2009). In human adults, it was demonstrated that the faecal metaproteome is enriched in proteins belonging to the COG categories involved in translation, energy production and carbohydrate metabolism (Verberkmoes et al. 2009). Compared with metatranscriptomics and metaproteomics approaches, metabolomic approaches have up to this date been applied more frequently, using NMR spectroscopy and mass spectroscopy in conjunction with computational multivariate analysis (Nicholson and Lindon 2008). A recent study by Martin and colleagues demonstrates that metabolic profiling can be used for studying nutrient-microbiota relations by examining the effects of dietary intervention on the presence of faecal metabolites (Martin et al. 2010). A variety of systemic diseases such as hypertension (Holmes et al. 2008) and diabetes (Dumas et al. 2007) appear to be directly influenced by microbial metabolism in model animals and human (Kinross et al. 2011). The metabolic pathways that are involved in drug metabolism are also influenced by the intestinal microbiota in an *in vitro* system (Aura et al. 2011).

Altogether, functional microbiomic approaches can be applied to examine microbial gene expression and to establish the effects of microbial gene products on the host. However, up to this date it is difficult to connect functionality to the presence of individual microbial species in the human GI tract. In order to link specific sets of genes to the presence of distinct microbial species, complete microbial genome sequences will be needed. Several independent research consortia have taken up the effort to sequence the genomes of hundreds of bacterial strains, which together will form a catalogue of reference genomes from the human microbiota. Recently, the initial sequencing of 178 reference genomes was reported and the first results of comparative genomic analysis of these sequences provided important insight into the inter-strain diversity of bacterial genomes (Nelson et al. 2010). Large-scale functional microbiomic analyses are needed to fully understand the impact of the human microbiome on the host. This means that a larger number of samples, deeper sequencing, longer sequence reads and more extensive comparative analyses are needed. Integration of all these microbiomic approaches will help to define the functional contribution of each individual microbial phylotype in the human GI tract to the health status of the host.

Changes in composition and diversity of the intestinal microbiota are related to disease

The type and number of microbial species that persist and colonise the GI tract is not determined by chance, but by a

combination of factors including but not limited to the inflammatory state of the host, diet, host genetics, and environmental factors (Buddington and Sangild 2011; Cerf-Bensussan and Gaboriau-Routhiau 2010; Hansen et al. 2010; Musso et al. 2010). This means that the host itself influences the composition of the intestinal microbiota. However, the relative impact of these factors on the intestinal microbiota is still largely unknown.

The intestinal microbiota and the host have co-evolved (Ley et al. 2008). Human evolution has taken place amidst a world of microorganisms. Symbiotic microorganisms have occupied the niches offered by the gastrointestinal tract and probably adapted to the local circumstances. This in turn may have influenced human evolution in terms of metabolic and nutritional requirements. Ultimately, man depends on its intestinal microbiota for a number of vital functions and thus these intestinal microorganisms may contribute to health. It is, however, difficult to describe the precise impact of the intestinal microbiota on human health and the involvement in human disease.

Perturbation of the microbiota composition, also known as dysbiosis, has been recognised in various diseases, of which many are associated with the GI tract. However, before dysbiosis can be established, the composition of a healthy ‘normal’ microbiota has to be defined. Yet, the definition of a healthy microbiota is not easy to give. From an operational point of view it could be stated that a healthy intestinal microbiota is the microbiota composition as it can be found in healthy individuals. For practical reasons, the phylogenetic characterisation of the microbiota of diseased individuals in comparison with apparently healthy individuals is, at this moment, the main approach to study changes in composition of the intestinal microbiota in relation to disease. However, since there are substantial inter-individual and intra-individual variations in the composition of the intestinal microbiota, it is difficult to establish the precise relations between human health and the presence and relative abundance of specific microbial communities. In the future, specific changes in compositional diversity, or even functional diversity, may be applied as biomarkers for health or specific diseases. It must be noted, however, that it is questionable whether changes in phylogenetic composition are really cause or consequence of a given disease.

A role for the intestinal microbiota in the pathogenesis of several diseases and disorders has been suggested. Intensively studied examples for which dysbiosis of the intestinal microbiota has been described, include inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and obesity, which will be discussed in-depth in this part of the review.

The microbial composition at different stages of life and its relation to health

The intestinal microbiota of healthy adult individuals is relatively stable over time (Costello et al. 2009; Franks et al. 1998; Vanhoutte et al. 2004; Zoetendal et al. 1998). However, intra-individual fluctuations occur due to environmental changes and pathological events. In addition, substantial changes in the composition of intestinal microbiota occur at both ends of life, in infants and elderly individuals (Tiihonen et al. 2010; Vael and Desager 2009). Since alterations in the microbial composition are recognised to be of influence on human health, the interest in the development and composition of the microbiota of infant and elderly humans has significantly increased in the last years.

It is widely accepted that microbial colonisation of the GI tract starts during and directly after birth when neonates are exposed to bacteria that are derived from the mother and the surrounding environment (Adlerberth and Wold 2009; Mackie et al. 1999). Yet, the human foetal environment is not completely microbiologically sterile and there are indications that non-pathological in utero exposure of the foetus to intestinal bacteria or bacterial DNA frequently occurs (Pettker et al. 2007; Satokari et al. 2009). In addition, the isolation of bacteria from the meconium (the first stool of the neonate), umbilical cord blood and amniotic fluid of healthy neonates has been reported (Jiménez et al. 2005, 2008). Postnatal colonisation of the GI tract is highly variable amongst neonates and is influenced by several factors including mode of delivery, type of infant feeding, gestational age, infant hospitalisation and antibiotic use (Penders et al. 2006). It is, however, still unclear how each of these factors exactly influences the infant microbial diversity and how this is related to health. A disturbed development of the infant microbiota has been associated with the development of disease later in life (Vael and Desager 2009). For example, associations have been made between dysbiosis in infants and the later development of childhood obesity (Collado et al. 2008b; Kalliomäki et al. 2008) and atopic and allergic diseases (Björkstén et al. 2001; Kalliomäki et al. 2001; Penders et al. 2007; Sjögren et al. 2009; Wang et al. 2008).

Several culture-independent studies have shown that there is a large inter-individual variability amongst infants in the development of the microbiota (Favier et al. 2002; Palmer et al. 2007; Penders et al. 2006; Roger et al. 2010). In addition, it has been demonstrated that the infant microbiota is highly dynamic and develops in a step-wise fashion with an increase in diversity over time (Palmer et al. 2007; Roger et al. 2010). An important stage in the colonisation of the GI tract of infants is the period in which the infants feed on the milk they receive either by

breastfeeding or by infant-formula feeding. During this period, the faecal microbiota of infants consists mainly of bifidobacteria (Roger and McCartney 2010; Roger et al. 2010). Some bifidobacteria are highly adapted to the digestion of the oligosaccharides present in human milk (Zivkovic et al. 2010). The infant intestinal microbiota contains a relatively low diversity in *Bifidobacterium* populations; *B. breve*, *B. bifidum* and *B. longum* subsp. *infantis* are the most common *Bifidobacterium* species (Roger et al. 2010). Compared with breast-fed infants, the intestinal microbiota of formula-fed infants is characterised by less diverse *Bifidobacterium* populations (Roger et al. 2010) and more complex communities of *Clostridia*, *Enterobacteriaceae*, *Bacteroides* and *Enterococcus* (Harmsen et al. 2000; Penders et al. 2006). The introduction of solid food (weaning) marks an increase in microbial diversity and changes in the microbial composition towards an adult microbiota (Koenig et al. 2010). For example, dominant *Bifidobacterium* populations change; *B. adolescentis*, *B. catenulatum* and *B. longum* subsp. *longum* are more abundantly present in the adult microbiota (Matsuki et al. 2004). The successive shifts of different microbial communities within the first years of life ultimately result in the development of an adult-like microbiota.

In the elderly (usually defined as people over the age of 65), there are major physiological changes that have an impact on the composition and the functionality of the intestinal microbiota (Tiihonen et al. 2010; Woodmansey 2007). Many elderly humans suffer from decreased intestinal motility, which can result in prolonged intestinal transit time and faecal retention. Age-related changes, such as decreased senses for smell and taste, dental decay and swallowing difficulties can lead to narrowing of the nutritional intake and even malnutrition. In addition, the age-related gradual deterioration of the immune system (immunosenescence) is associated with changes in intestinal microbiota composition (Schiffrin et al. 2010). Furthermore, the increased use of laxatives, antibiotics and other medication in elderly individuals will affect intestinal microbiota composition.

Culture-independent studies have demonstrated that the composition of the intestinal microbiota significantly changes with age (Bartosch et al. 2004; Mariat et al. 2009; Mueller et al. 2006; Zwielehner et al. 2009). Recently, high-throughput methods have been applied to study the changes in the intestinal microbiota of elderly individuals. Biagi and colleagues have used the HITChip, a phylogenetic microarray specifically designed to study the human GI tract microbiota, to compare the intestinal microbiota composition of young adults with that of elderly individuals and centenarians. It was demonstrated that especially the microbiota of centenarians showed significant differences compared with microbiota composition of the other

two age groups (Biagi et al. 2010). The microbiota of centenarians was characterised by low species-level diversity, specific changes in *Firmicutes* subpopulations, enrichment in *Proteobacteria* and a decrease in bifidobacteria. In addition, high-throughput next generation sequencing has been used by Claesson and colleagues to study the composition, variability and temporal stability of the intestinal microbiota of the elderly (Claesson et al. 2010). They observed that the faecal microbiota of elderly individuals was relatively stable over a 3 month period in the majority of the subjects. However, compared with younger control subjects, the microbiota of the elderly was characterised by a high inter-individual variation in microbiota composition, also at phylum level. The relative abundance of the *Firmicutes* varied between 8% and 80%, whereas the *Bacteroidetes* levels varied between 14% and 92%. Furthermore, it was found that in the majority of the elderly subjects the microbiota was characterised by a higher *Bacteroidetes/Firmicutes* ratio compared with that observed in younger adults. In addition, distinct differences were seen in *Proteobacteria*, *Actinobacteria* and *Clostridium* populations between young and older adults. With respect to human health, associations have been found between microbiota composition and frailty in elderly individuals (Van Tongeren et al. 2005). In frail elderly persons, a significant reduction in the number of lactobacilli, *Faecalibacterium prausnitzii* and *Bacteroides-Prevotella* groups was seen. In contrast, the number of *Enterobacteriaceae* was significantly higher. The number of studies that have focused on the age-related differences in intestinal microbiota composition is still limited. In general, they suggest that maintenance of (microbial) homeostasis in the GI tract is essential for healthy ageing.

Microbial diversity and IBS

IBS is a functional bowel disorder which is characterised by recurrent abdominal pain or discomfort, irregular bowel movements and disordered stool patterns such as constipation or diarrhoea (Longstreth et al. 2006). The occurrence of these symptoms, however, can vary from person to person. The aetiology of IBS is probably complex and still not well understood. Several factors are thought to be involved in IBS and may include altered GI motility, visceral hypersensitivity, low-grade inflammation, and psychosocial (anxiety and depression), genetic and dietary factors (Chang and Talley 2011; Karantanos et al. 2010).

Several studies, using both culture-dependent and independent methods, have demonstrated an association between IBS and dysbiosis of the intestinal microbiota (Table 2). In general, faecal material has been used to study dysbiosis in IBS patients. However, more recently also duodenal (Kerckhoffs et al. 2009, 2010) and colonic

(Carroll et al. 2010; Codling et al. 2010) biopsies have been used to study the mucosa-associated microbiota. Most studies aimed to show changes in the predominant microbial communities, however, in some cases the focus was more on specific microbial groups. Quantitative differences in microbiota composition, even for specific microbial groups, have been observed in IBD patients compared with healthy individuals. However, the results of the various studies are inconsistent and no consensus has been reached on the association between specific microbial groups and IBS, notably as the power of the studies was low and the depth of the analysis was limited (Salonen et al. 2010a). At the functional level, some studies have demonstrated altered colonic fermentation patterns and increased gas production in IBS patients (Koide et al. 2000; Mortensen et al. 1987; Tana et al. 2010; Treem et al. 1996). However, these results have not yet been confirmed in other studies at the molecular level.

In most of the studies, IBS patients have been classified into different subtypes based on Rome II criteria for IBS: diarrhoea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C) or a mixed type of IBS with alternating stool patterns (IBS-A). Distinct changes in microbiota composition have been observed in the different IBS subtypes compared with healthy individuals (Malinen et al. 2005; Maukonen et al. 2006; Lyra et al. 2009). It appears that the intestinal microbiota of IBS-D patients deviates the most from that of healthy individuals (Carroll et al. 2010; Krogius-Kurikka et al. 2009). These data demonstrate the relevance of clinical subtyping of IBS patients when analysing the intestinal microbiota. So far, however, the results from the studies which have applied IBS subtyping have also not shown uniform changes in microbial composition (Salonen et al. 2010a).

Microbial diversity and IBD

IBD is a collective name for chronic inflammatory disorders of the GI tract, of which Crohn's disease (CD) and ulcerative colitis (UC) are the most prevalent forms. These are both chronic and relapsing diseases that affect the intestinal mucosa. For both CD and UC, the exact aetiology is still not clear, however, it has been suggested that an aberrant immune response directed against intestinal microbial antigens is involved (Hansen et al. 2010; Sartor 2008; Sokol and Seksik 2010). CD affects the whole GI tract and is characterised by discontinuous inflammation of the epithelial lining and deep ulcers. UC on the other hand is restricted to the colon and the rectum and is characterised by a continuous mucosal inflammation and superficial ulcers.

During the last decade, numerous culture-independent studies have compared the intestinal microbiota composition of IBD patients with that of healthy individuals

Table 2 Overview of human studies that demonstrate an association between IBS and compositional dysbiosis of the intestinal microbiota determined with culture-independent methods

Study material	Population	Analytical methods	References
Faeces (3 time points)	27 IBS patients 22 Healthy individuals	qPCR	Malinen et al. (2005)*
Faeces (3 time points)	26 IBS patients 25 Healthy individuals	Conventional culturing DGGE Clone library sequencing (16S)	Mättö et al. (2005)*
Biopsies: inflamed and non-inflamed tissue (ileum, ascending/sigmoid colon)	20 CD patients 20 UC patients 20 Self-limiting colitis patients 20 IBS patients 20 Healthy individuals	FISH	Swidsinski et al. (2005)
Faeces (2 time points)	16 IBS patients 16 Healthy individuals	DGGE TRAC	Maukonen et al. (2006)*
Faeces	24 IBS patients 23 Healthy individuals	G+C based profiling Clone library sequencing (16S) qPCR	Kassinen et al. (2007)*
Duodenal biopsies	41 IBS patients	FISH	Kerckhoffs et al. (2009)#
Faeces	26 Healthy individuals	qPCR	
Faeces	10 (+2) IBS (only IBS-D) 23 Healthy individuals	G+C based profiling Clone library sequencing (16S) qPCR	Krogus–Kurikka et al. (2009)*
Faeces (3 time points)	20 IBS patients 15 Healthy individuals	qPCR	Lyra et al. (2009)*
Colonic biopsies	10 IBS patients (only IBS-D)	Conventional culturing	Carroll et al. (2010)
Faeces	10 Healthy individuals	qPCR	
Colonic biopsies	47 IBS patients	DGGE	Codling et al. (2010)
Faeces	33 Healthy individuals		
Duodenal biopsies	37 IBS patients	DGGE	Kerckhoffs et al. (2010)#
Faeces	20 Healthy individuals	Clone library sequencing (16S) qPCR	
Faeces	44 IBS patients	qPCR	Malinen et al. (2010)*
Faeces	26 IBS patients 26 Healthy individuals	Conventional culturing qPCR HPLC	Tana et al. (2010)

All studies have applied Rome II or III criteria to recruit their subjects and categorise them in IBS subtypes. Studies that have used subjects from the same cohort are indicated by * and #

DGGE denaturing gradient gel electrophoresis, FISH fluorescence in situ hybridisation, HPLC high-performance liquid chromatography, qPCR quantitative polymerase chain reaction, TRAC transcript analysis with the aid of affinity capture

(Table 3). There is increasing evidence that dysbiosis of the intestinal microbiota has a role in the pathogenesis of IBD. Up to this date, however, the phylum-level changes observed in IBD patients have not always been consistent. In general, an overall decrease in microbial diversity and stability of the intestinal microbiota has been observed in IBD patients (Hansen et al. 2010). In addition, a decrease in specific members of the *Firmicutes* has been reported in IBD patients, which in some cases coincided with an increase in *Bacteroidetes* and facultative anaerobes such as

Enterobacteriaceae (Hansen et al. 2010). Significant differences exist in the microbiota composition of CD patients compared with UC patients (Frank et al. 2007; Sokol et al. 2006). Recently, Joossens and colleagues identified a set of five bacterial species that characterised the predominant dysbiosis in CD patients compared with unaffected relatives and healthy individuals (Joossens et al. 2011). These five species are *Dialister invisus*, an uncharacterised species of *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis* and *Ruminococcus*

Table 3 Overview of human studies that demonstrate an association between IBD and compositional dysbiosis of the intestinal microbiota determined with culture-independent methods

Study material	Population	Analytical methods	References
Biopsies (terminal ileum, colon)	12 CD patients (active disease) 12 UC patients (active disease) 14 Non-IBD controls	FISH	Kleessen et al. (2002)
Biopsies: inflamed and non-inflamed tissue (ileum, ascending/sigmoid colon)	28 Self-limiting colitis patients 104 Indeterminate colitis patients 119 UC patients 54 CD patients 40 Non-IBD controls	Conventional culturing qPCR FISH	Swidsinski et al. (2002)
Faeces	8 CD patients (active disease) 9 CD patients (in remission) 16 Healthy individuals	Dot-blot hybridisation TGGE	Seksik et al. (2003)
Faeces	4 CD patients 4 Healthy controls	Clone library sequencing (16S)	Mangin et al. (2004)
Colonic biopsies: inflamed tissue	26 CD patients (active disease) 31 UC patients (active disease) 15 Inflammatory controls 31 Non-inflammatory controls	SSCP Clone library sequencing (16S) qPCR	Ott et al. (2004)
Rectal biopsies: inflamed and non-inflamed tissue	4 CD patients (active disease) 2 CD patients (in remission) 14 UC patients (active disease) 19 UC patients (in remission) 14 Non-IBD controls	FISH	Mylonaki et al. (2005)
Biopsies: inflamed and non-inflamed tissue (ileum, ascending/sigmoid colon)	20 CD patients 20 UC patients 20 Self-limiting colitis patients 20 IBS patients 20 Non-IBD controls	FISH	Swidsinski et al. (2005)
Biopsies: inflamed and non-inflamed tissue (ileum, ascending/transverse/descending colon, rectum)	20 CD patients (active disease) 15 UC patients (active disease) 14 Non-IBD controls	Clone library sequencing (16S) DGGE qPCR	Bibiloni et al. (2006)
Biopsies: inflamed and non-inflamed tissue (ileum, transverse/sigmoid colon, rectum)	6 CD patients 5 UC patients 5 Non-IBD controls	Clone library sequencing (16S)	Gophna et al. (2006)
Faeces	6 CD patients (in remission) 6 Healthy individuals	Clone library screening (metagenome: 16S) FISH/flow cytometry	Manichanh et al. (2006)*
Biopsies: inflamed and non-inflamed tissue (ileum, ascending/transverse/descending/sigmoid colon, rectum)	19 CD patients 2 UC patients 1 Ischemic colitis patient 15 Non-IBD controls	DGGE 16S rRNA gene sequence analysis	Martinez–Medina et al. (2006)
Faeces (several time points)	16 CD patients 18 Healthy individuals	DGGE	Scanlan et al. (2006)

Table 3 continued

Study material	Population	Analytical methods	References
Faeces	13 CD patients (active disease) 13 UC patients (active disease) 5 Infectious colitis patients 13 Healthy individuals	FISH/flow cytometry	Sokol et al. (2006) [#]
Faeces	29 UC patients (active disease) 12 UC patients (in remission) 46 Healthy individuals	T-RFLP	Andoh et al. (2007)
Ileal biopsies	13 CD patients (ileum) 8 CD patients (colon) 7 Non-IBD controls	Clone library sequencing (16S) qPCR FISH	Baumgart et al. (2007)
Biopsies (small intestine and colon)	68 CD patients 61 UC patients 61 Non-IBD controls	Clone library sequencing (16S) qPCR	Frank et al. (2007)
Biopsies (caecum, colon, rectum)	13 CD patients 19 UC patients 15 Healthy individuals	RISA Conventional culturing	Kotlowski et al. (2007)
Faeces	17 CD patients (active disease) 17 CD patients (in remission) 20 Healthy controls	T-RFLP	Andoh et al. (2008)
Faeces	10 Twin pairs with CD 8 Healthy twin pairs	G+C profiling Clone library sequencing (16S) T-RFLP	Dicksved et al. (2008) [†]
Faeces (several time points)	16 UC patients (in remission) 8 Healthy controls	DGGE	Martinez et al. (2008)
Colonic biopsies Faeces	15 CD patients (active disease) 8 CD patients (in remission) 44 UC patients (active disease) 29 UC patients (in remission)	Conventional culturing qPCR FISH HPLC	Takaishi et al. (2008)
Rectal biopsies: inflamed and non-inflamed tissue	9 UC patients (active disease) 11 Non-IBD controls	T-RFLP	Nishikawa et al. (2009)
Faeces	22 CD patients (active disease) 10 CD patients (in remission) 12 UC patients (active disease) 4 UC patients (in remission) 8 Infectious colitis patients 27 Healthy individuals	qPCR	Sokol et al. (2009) [#]
Biopsies (terminal ileum, ascending/transverse/descending colon, rectum)	10 Twin pairs with CD 8 Healthy twin pairs	T-RFLP Clone library sequencing (16S) qPCR	Willing et al. (2009) [†]
Faeces	6 CD patients (in remission) 6 Healthy individuals	qPCR Phylogenetic microarray (16S)	Kang et al. (2010) [*]

Table 3 continued

Study material	Population	Analytical methods	References
Faeces	4 CD patients (in remission) 21 UC patients (in remission) 14 Healthy individuals	Clone library sequencing (metagenome)	Qin et al. (2010)
Faeces	68 CD patients (in remission) 84 Unaffected relatives 55 Healthy individuals	DGGE qPCR	Joossens et al. (2011)
Faeces	16 CD patients (active disease) 16 Healthy individuals	qPCR Phylogenetic microarray (16S)	Mondot et al. (2011)
Biopsies: inflamed and non-inflamed tissue (ileum, ascending/transverse/ descending/sigmoid colon, rectum)	12 CD patients (active disease) 6 UC patients (active disease) 5 Non-IBD controls	qPCR Clone library sequencing (16S)	Walker et al. (2011)

Most of the studies used the Crohn's disease activity index (CDAI; for CD) and/or the clinical activity index (CAI; for UC patients) to assess disease activity in the subjects and to define active disease or remission. Studies that have used subjects from the same cohort are indicated by *, # and †

DGGE denaturing gradient gel electrophoresis, FISH fluorescence in situ hybridisation, HPLC high-performance liquid chromatography, qPCR quantitative polymerase chain reaction, RISA ribosomal intergenic spacer analysis, SSCP single strand conformation polymorphism, T-RFLP terminal-restriction fragment length polymorphism, TGGE temperature gradient gel electrophoresis

gnavus. Of these species, *F. prausnitzii* has been associated with prolongation of remission in CD (see also below and Sokol et al. 2008, 2009), while bifidobacteria in general have shown to have beneficial effects on health (see above). Most interestingly, the unaffected relatives of CD patients also have a different composition of their predominant microbiota compared with healthy individuals in general. The impact of these observations on IBD diagnostics and aetiology now has to be addressed.

The role of several different microorganisms in the aetiology of IBD has been studied in more detail. Adherent-invasive *Escherichia coli* (Darfeuille-Michaud 2002; Darfeuille-Michaud et al. 2004) and *Mycobacterium avium* subspecies *paratuberculosis* (Rosenfeld and Bressler 2010) are two prime suspects that have been implicated to be involved in CD pathogenesis. However, a causal relation has not yet been demonstrated. Recently, the presence of two species belonging to the family *Enterobacteriaceae*, *Klebsiella pneumoniae* and *Proteus mirabilis*, was correlated with the development of colitis in a mouse model (Garrett et al. 2010). The evidence that specific microorganisms can induce intestinal inflammation and cause IBD is, however, still inconclusive, despite the considerable amount of studies concerning this subject. In addition to the identification of potential pathogenic bacteria, other bacterial species have been suggested to protect against IBD. For example, it has been shown that the relative abundance of *F. prausnitzii*, a commensal bacterium with anti-inflammatory properties, is significantly decreased in CD patients compared with healthy individuals (Sokol et al. 2008).

High-throughput metagenomic studies can provide more insight in the composition and diversity of the intestinal microbiota of IBD patients. IBD is amongst the first diseases that have been the subject of metagenomic investigation (Qin et al. 2010). Based on the relative abundance of 155 microbial species (present in at least one individual at a genome coverage of $\geq 1\%$ in this study population), it was possible to separate patients from healthy individuals, and UC from CD patients (Qin et al. 2010). The next step is to compare the IBD subpopulations with healthy individuals at microbial gene-level. On average, 25% fewer genes could be detected in the faecal samples of IBD patients compared with individuals not suffering from IBD (Qin et al. 2010). These results suggest that the microbiota of IBD patients has a lower functional diversity compared with healthy individuals. The intestinal microbiota in IBD patients produce reduced amounts of SCFA, in particular butyrate, while sulphate reduction (by sulphate-reducing bacteria) is increased (Fava and Danese 2011). In the near future, metagenomic studies like these will provide more insight in the shifts in functionality which characterises the differences between IBD patients and healthy individuals.

The observed compositional and functional changes in IBD patients suggest that the intestinal microbiota plays an important role in the aetiology and pathogenesis of IBD. However, up to this date it is still unclear whether dysbiosis is a direct cause for the inflammation in IBD, or merely the result of a disturbed environment in the GI tract. In the latter case, a role for the intestinal microbiota in disease

maintenance and severity is possible and will have to be explored in the future.

Microbial diversity and obesity

Obesity is a complex disease characterised by excess body fat accumulation. It has been associated with phylum-level changes in the composition of the intestinal microbiota (Table 4). An increase in the relative abundance of *Firmicutes* and a reduction in the level of *Bacteroidetes* has been observed in both obese mice (*ob/ob*) (Ley et al. 2005) and humans (Ley et al. 2006). However, since the original publication, a series of studies have failed to confirm the study of Ley and colleagues and shown variable results with respect to the compositional changes in the microbiota of obese humans (Collado et al. 2008b; Duncan et al. 2008; Kalliomäki et al. 2008; Nadal et al. 2009; Santacruz et al. 2009, 2010; Schwartz et al. 2010; Zhang et al. 2009). Altogether these data suggest that instead of phylum-level changes, more subtle changes in the composition of the intestinal microbiota are associated with the development of obesity. Recently, Turnbaugh and colleagues have observed a reduced compositional microbial diversity in obese individuals compared with lean individuals (Turnbaugh et al. 2009).

It is evident that (excessive) food intake has an influence on body (over)weight. Recently, a direct link between intestinal microbiota composition and body weight has been suggested. One of the first publications that provides evidence for this link is the publication by Bäckhed and colleagues for which they colonised germ-free mice with the microbiota of conventionally raised mice (Bäckhed et al. 2004). They observed an increase of body fat content of the colonised germ-free mice despite reduced food intake, which was suggested to be caused by the introduction of intestinal microbial communities. In a later study, it was demonstrated that the absence of intestinal microorganisms protected germ-free mice against the development of obesity after being fed a high-fat, sugar-rich diet (Bäckhed et al. 2007). Furthermore, it was demonstrated that colonisation of germ-free mice with the microbiota of obese mice induced a significant greater increase in body fat weight compared with germ-free mice colonised with the microbiota of lean mice (Turnbaugh et al. 2006). In addition, these experiments in germ-free mice have demonstrated that the intestinal microbiota is involved in the regulation of fat storage. It was shown that introduction of an intestinal microbiota resulted in an increase in metabolic rate, modulation of de novo lipogenesis and an increase in the uptake of monosaccharides from the intestine (Bäckhed et al. 2004). Based on these results, it has been hypothesised that obese individuals are more efficient in converting food into usable energy and in

storing this energy in fat than lean individuals (Turnbaugh et al. 2006). As discussed above, the intestinal microbiota has a crucial role in the digestion of food, in particular the metabolism of polysaccharides and oligosaccharides and the production of SCFA that provide the host with additional amounts of energy. Altered representation of bacterial genes and metabolic pathways, including those involved in nutrient harvest, were found to be related to obesity (Turnbaugh et al. 2009). The results from this study demonstrate that major insights in the differences between various physiological states of the host (in this case obese vs. lean) can be obtained by studying the functional microbial diversity in addition to phylogenetic diversity. In line with this conclusion is the observation that the amount of SCFA produced by the intestinal microbiota rather than the changes in the composition of the microbiota are important in the development of obesity (Schwartz et al. 2010).

As for IBD and IBS, which were discussed above, also for obesity the question remains whether dysbiosis of the intestinal microbiota is a direct cause for obesity or whether it reflects a disturbed host environment. It needs to be established whether the changes in the intestinal microbial communities in obese individuals are not merely an adaptation to a change in the host's diet. Some of the studies that have shown an altered composition of the intestinal microbiota in obese individuals, have also incorporated analysis of the effect of diet change on the observed dysbiosis (Table 4) (Duncan et al. 2007, 2008; Ley et al. 2006; Nadal et al. 2009; Santacruz et al. 2009). Little is known, however, about the influence of dietary change on microbiota composition in humans. A recent study demonstrated rapid and reversible changes in the relative abundance of specific dominant bacterial groups after dietary changes (Walker et al. 2011a). Most striking was the strong increase in the relative abundance of *Ruminococcus bromii* and *Eubacterium rectale* phylotypes as result of a diet rich in resistant starch. It was suggested that indigestible dietary polysaccharides can substantially change the composition of the intestinal microbiota, however, it is likely that this depends on the initial composition of the intestinal microbiota. Interestingly, *R. bromii* and *E. rectale* were identified as key degraders of starch in an in vitro model of the human colon, using 16S rRNA-based stable isotope probing (Kovatcheva-Datchary et al. 2009). Recent studies in mice show that the influence of the diet (high-fat vs. standard chow or low-fat) on the composition of the intestinal microbiota is independent of genetic disposition for obesity (Hildebrandt et al. 2009; Murphy et al. 2010).

In addition to obesity, it has also been suggested that the intestinal microbiota is involved in obesity-associated metabolic disorders, such as type 2 diabetes metabolic endotoxemia, low-grade inflammation and adiposity (Cani

Table 4 Overview of human studies that demonstrate an association between obesity and compositional dysbiosis of the intestinal microbiota determined with culture-independent methods

Study material	Population	Analytical methods	Key findings	References
Faeces (3 time points)	12 Obese individuals (on diet) 2 Normal-weight individuals	Clone library sequencing (16S)	Obese individuals compared with lean: ↓ <i>Bacteroidetes</i> ↑ <i>Firmicutes</i>	Ley et al. (2006)
Faeces (3 time points)	19 Obese individuals (on diet)	FISH GC	Obese individuals on diet of decreased carbohydrate intake: ↓ <i>Roseburia</i> ↓ <i>Eubacterium rectale</i> subgroup of cluster XIVa ↓ bifidobacteria	Duncan et al. (2007)*
Faeces (2 time points)	18 Obese pregnant women 36 Normal-weight pregnant women	FISH/flow cytometry qPCR	Overweighed pregnant women: ↑ <i>Bacteroides</i> ↑ <i>Clostridium</i> ↑ <i>Staphylococcus</i>	Collado et al. (2008b) [#]
Faeces (3 time points)	23 Overweight/obese individuals (on diet) 14 Non-obese individuals	FISH	During weight-loss diet: ↔ <i>Bacteroidetes</i> ↓ butyrate-producing <i>Firmicutes</i>	Duncan et al. (2008)*
Faeces (2 time points)	25 Overweight/obese children 24 Normal-weight children (prospective study)	FISH/flow cytometry qPCR	Intestinal microbiota during infancy preceding overweight during childhood: ↓ bifidobacteria ↑ <i>Staphylococcus aureus</i>	Kalliomäki et al. (2008)
Faeces	20 Obese individuals 9 Individuals with anorexia nervosa 20 Normal-weight individuals	qPCR	Obese individuals: ↓ <i>Bacteroidetes</i> ↑ <i>Lactobacillus</i> Anorexic individuals: ↑ <i>Methanobrevibacter smithii</i>	Armougom et al. (2009)
Faeces (2 time points)	39 Overweight/obese adolescents (on diet and physical activity)	FISH/flow cytometry	Obese individuals: ↑ <i>C. histolyticum</i> ↑ <i>E. rectale-C. coccoides</i> Upon calorie restricted diet: ↓ <i>C. histolyticum</i> ↓ <i>C. lituseburensis</i> ↓ <i>E. rectale-C. coccoides</i> ↑ <i>Bacteroides-Prevotella</i> group	Nadal et al. (2009) [†]
Faeces (2 time points)	36 Overweight/obese adolescents (on diet and physical activity)	qPCR	Obese adolescents on diet with a high weight-loss: ↑ Total bacteria ↑ <i>B. fragilis</i> group ↑ <i>C. leptum</i> group ↑ <i>B. catenulatum</i> group ↓ <i>C. coccoides</i> group ↓ <i>Lactobacillus</i> group	Santacruz et al. (2009) [†]

Table 4 continued

Study material	Population	Analytical methods	Key findings	References
Faeces (2 time points)	31 Monozygotic twin pairs 23 Dizygotic twin pairs 46 Mothers of twin pairs	Sanger sequencing (16S) 454 FLX titanium sequencing (metagenome)	Most obesity-associated genes are from: <i>Actinobacteria</i> <i>Firmicutes</i> Most lean-enriched genes are from <i>Bacteroidetes</i>	Turnbaugh et al. (2009)
Faeces	3 Obese individuals 3 Individuals with a gastric-bypass 3 Normal-weight individuals	Clone library sequencing (16S) 454 FLX titanium sequencing (16S) qPCR	Obese individuals: ↑ H ₂ -producing <i>Prevotellaceae</i> ↑ H ₂ -utilizing methanogenic <i>Archaea</i>	Zhang et al. (2009)
Faeces	15 Obese Indian adolescents 13 Non-obese Indian adolescents	qPCR	Obese children: ↔ <i>Bacteroides-Prevotella</i> ↔ <i>Bifidobacterium</i> ↔ <i>L. acidophilus</i> ↔ <i>E. rectale</i> ↑ <i>F. prausnitzii</i>	Balamurugan et al. (2010)
Faeces (2 time points)	16 Infants of overweight women 26 Infants of normal-weight women	FISH/flow cytometry qPCR	Infants of overweight mothers: ↑ <i>Bacteroides</i> ↑ <i>Staphylococcus</i>	Collado et al. (2010) [#]
Faeces	33 Obese individuals 35 Overweight individuals 30 Normal-weight individuals	qPCR GC	Obese individuals compared with lean: ↑ <i>Bacteroidetes</i> ↓ <i>Firmicutes</i>	Schwartz et al. (2010)
Faeces	16 Overweight pregnant women 34 Normal-weight pregnant women	qPCR	Overweight pregnant women: ↓ <i>Bifidobacterium</i> ↓ <i>Bacteroides</i> ↑ <i>Staphylococcus</i> ↑ <i>Enterobacteriaceae</i> ↑ <i>E. coli</i>	Santacruz et al. (2010)

All studies have used the body mass index (BMI) to define normal weight, overweight and obesity. Studies that have used subjects from the same cohort are indicated by *, # and †

FISH fluorescence in situ hybridisation, *GC* gas chromatography, *qPCR* quantitative polymerase chain reaction

and Delzenne 2009; Ley 2010; Vrieze et al. 2010). In a recent study, a high-throughput sequencing approach was used to demonstrate that type 2 diabetes, a metabolic disease primarily caused by obesity-linked insulin resistance, is associated with changes in the composition of the intestinal microbiota (Larsen et al. 2010). The relative abundance of *Firmicutes* was significantly lower in diabetic patients compared with non-diabetic persons. On the other hand, the *Bacteroidetes* and *Proteobacteria* were present in higher abundance. In addition, the *Bacteroidetes/Firmicutes* and *Bacteroides-Prevotella/C.coccoides-E.rectale* ratios were positively and significantly correlated with plasma glucose levels.

Microbial diversity and other human diseases

In addition to IBD, IBS and obesity, the intestinal microbiota has also been suggested to be involved in several other (chronic) diseases and disorders. Associations have been described between intestinal microbial dysbiosis and intestinal diseases such as coeliac disease, colorectal cancer, pouchitis and necrotizing enterocolitis (NEC) (Table 5). The most recent data show that the intestinal microbiota of coeliac disease patients displays a greater diversity than healthy controls with higher numbers of *Bacteroides-Prevotella* (De Palma et al. 2010; Schippa et al. 2010). *Bifidobacterium*, *Clostridium histolyticum*, *C.*

Table 5 Overview of human studies that demonstrate an association between intestinal disease and compositional dysbiosis of the intestinal microbiota

Study material	Population	Analytical methods	Reference
<i>Coeliac disease</i>			
Faeces	26 Coeliac patients (no diet, active disease)	Conventional culturing	Collado et al. (2007)
	23 Children without gluten intolerance	FISH	
Duodenal biopsies	20 Coeliac patients (no diet, active disease)	FISH/flow cytometry	Nadal et al. (2007)
	10 Coeliac patients (gluten-free diet, symptom-free)		
	8 Children without gluten intolerance		
Faeces	10 Coeliac patients (no diet, active disease)	DGGE	Sanz et al. (2007)
	10 Children without gluten intolerance		
Duodenal biopsies faeces	30 Coeliac patients (no diet)	qPCR	Collado et al. (2008a)
	18 Coeliac patients (gluten-free diet)		
	30 Children without gluten intolerance		
Faeces	24 Coeliac patients (no diet, active disease)	FISH/flow cytometry	De Palma et al. (2010)
	18 Coeliac patients (gluten-free diet, symptom-free)		
	20 Children without gluten intolerance		
Duodenal biopsies	20 Coeliac patients (active disease/ symptom-free)	TGGE	Schipa et al. (2010)
	10 Children without gluten intolerance		
<i>Colorectal cancer</i>			
Faeces	18 Patients with polyps	Conventional culturing	Moore and Moore (1995)
	32 Individuals with high -risk for colon cancer		
	38 Individuals with low-risk for colon cancer		
Faeces	13 Patients at high risk for sigmoid colon cancer	Conventional culturing	Kanazawa et al. (1996)
	14 Healthy individuals		
Faeces (3 time points)	20 Colon cancer patients	DGGE	Scanlan et al. (2008)
	20 Polypectomized patients		
	20 Healthy individuals		
Colorectal biopsies	21 Individuals with adenomas	T-RFLP	Shen et al. (2010)
	23 Individuals without adenomas	Clone library sequencing (16S)	
		FISH	
<i>Pouchitis</i>			
Pouch biopsies	12 Patients with pouchitis	Conventional culturing	Onderdonk et al. (1992)
Ileostomy effluent	14 Patients with indeterminable pouchitis		
Faeces	23 Patients without pouchitis		
	20 Ileostomy patients		
	9 Healthy individuals		
Pouch effluent	5 Patients with pouchitis	Conventional culturing	Ruseler-van Embden et al. (1994)
	9 Patients without pouchitis		
Pouch effluent	UC patients:	Conventional culturing	Ohge et al. (2005)
	8 Patients with healthy pouches		
	9 Patients, no active pouchitis for at least 1 year		
	9 Patients, no active pouchitis for at least 6 weeks		
	11 Patients with pouchitis, on antibiotic treatment		
	8 Patients with pouchitis		
	FAP patients:		
	5 Patients with healthy pouches		

Table 5 continued

Study material	Population	Analytical methods	Reference
Pouch effluent	9 Patients with pouchitis (UC) 13 Patients with healthy pouches (UC)	Conventional culturing	Iwaya et al. (2006)
Ileum biopsies	5 Patients with pouchitis (UC)	LH-PCR	Komanduri et al. (2007)
Pouch biopsies	15 Patients with healthy pouches (UC)	Clone library sequencing (16S)	
Pouch effluent	13 Healthy individuals		
Pouch effluent	5 Patients with pouchitis (UC) 15 Patients with healthy pouches (UC)	T-RFLP Clone library sequencing (16S)	Lim et al. (2009)
Pouch contents	9 Patients with pouchitis (UC)	T-RFLP	Zella et al. (2011)
Pouch biopsies	3 Patients with healthy pouches (UC) 7 Patients with healthy pouches (FAP)	Clone library sequencing (16S)	
<i>Necrotizing enterocolitis</i>			
Faeces	10 Preterm infants with NEC 10 Preterm infants without NEC	T-RFLP Clone library sequencing (16S)	Wang et al. (2009)
Faeces (several time points)	6 Preterm infants with NEC or suspected sepsis 6 Preterm control infants	DGGE 454 FLX titanium sequencing (16S)	Mshvildadze et al. (2010)

The intestinal diseases IBD, IBS and obesity are discussed separately in the article

DGGE denaturing gradient gel electrophoresis, FAP familial anastomosis polyposis, FISH fluorescence in situ hybridisation, LH-PCR length heterogeneity polymerase chain reaction, qPCR quantitative polymerase chain reaction, T-RFLP terminal-restriction fragment length polymorphism, TGGE temperature gradient gel electrophoresis, UC ulcerative colitis

lituseburensis and *F. prausnitzii* were less abundant in coeliac disease patients (De Palma et al. 2010). Also in the case of colorectal cancer, the bacterial diversity and richness was observed to be higher in patients compared with healthy controls (Shen et al. 2010). In addition, the intestinal microbiota composition of colorectal cancer patients differs from that of healthy controls, however, no consistent pattern has yet been observed.

The mucosal and faecal microbiota of UC pouchitis patients contained more *Clostridium* and *Eubacterium* and fewer *Lactobacillus* and *Streptococcus* genera compared with the microbiota of healthy pouches from familial adenomatous polyposis (FAP) patients (Lim et al. 2009; Zella et al. 2011). Luminal samples of UC pouchitis patients contained more *Firmicutes* and *Verrucomicrobia* and fewer *Bacteroidetes* and *Proteobacteria* compared with FAP patients.

The overall microbiota profiles of premature infants with necrotizing enterocolitis (NEC) were not distinguishable from that of control subjects, but 16S rRNA gene sequence analysis detected *Citrobacter*-like sequences and an increased frequency of *Enterococcus*-like sequences (Mshvildadze et al. 2010).

Intestinal microbial dysbiosis has also been observed in extra-intestinal diseases such as atopic and allergic diseases, autism, type 2 diabetes and rheumatoid arthritis (Table 6). In children who develop an allergic disease later in life, a reduced diversity of faecal microbiota was already

observed at 1 week of age (Wang et al. 2009; Niers et al., personal communication). During the first 2 months of life, they were less often colonised with lactobacilli group I (*L. rhamnosus*, *L. casei*, *L. paracasei*), *B. adolescentis* and *Clostridium difficile* (Kalliomäki et al. 2001; Sjögren et al. 2009).

The number of *Clostridium* species found in the stools of children with autism was greater than in the stools of control children, specifically of the *C. histolyticum* group (*Clostridium* clusters I and II) (Finegold et al. 2002; Paracho et al. 2005). *Bacteroidetes* was found at high levels in the severely autistic children while populations of the *Bifidobacterium* genus were reduced (Finegold et al. 2010).

Firmicutes and *Clostridia* are reduced in type 2 diabetes (Larsen et al. 2010). Furthermore, the *Bacteroidetes/Firmicutes* ratio as well as *Bacteroides-Prevotella/C. coccoides-E. rectale* ratio were observed to be correlated with plasma glucose concentration. In a Chinese population of diabetes patients, reduced populations of bifidobacteria were found (Wu et al. 2010).

In comparison to patients with fibromyalgia, patients with rheumatoid arthritis had significantly less bifidobacteria and bacteria of the *Bacteroides-Prevotella* group, *Bacteroides fragilis* subgroup, and *E. rectale-C. coccoides* group (Vaahtovuori et al. 2008).

Almost all of the diseases and disorders mentioned above are largely undefined and have a heterogeneous aetiology, which makes it difficult to relate changes in

Table 6 Overview of human studies that demonstrate an association between extra-intestinal disease and compositional dysbiosis of the intestinal microbiota

Study material	Population	Analytical methods	References
<i>Atopic and allergic diseases</i>			
Faeces	27 Allergic children 36 Non-allergic children	Conventional culturing	Björkstén et al. (1999)
Faeces (5 time points)	18 Infants who developed allergy 26 Infants who remained non-allergic	Conventional culturing	Björkstén et al. (2001)
Faeces (2 time points)	76 Infants at high risk for atopic disease	Conventional culturing FISH	Kalliomäki et al. (2001)
Faeces (2/3 time points)	27 Infants with atopic dermatitis 10 Infants without atopic dermatitis	Conventional culturing FISH	Kirjavainen et al. (2001)
Faeces	30 Children with atopic dermatitis 68 Children without atopic dermatitis	Conventional culturing	Watanabe et al. (2003)
Faeces	957 Infants	qPCR	Penders et al. (2007)
Faeces	20 Allergic children 20 Non-allergic children	DGGE	Štšepetova et al. (2007)
Faeces (3 time points)	10 Allergic infants 16 Non-allergic infants	qPCR	Suzuki et al. (2007)
Faeces	37 Infants with atopic dermatitis 24 Infants without atopic dermatitis	TGGE FISH/flow cytometry	Gore et al. (2008)
Faeces	15 Infants who developed atopic dermatitis 20 Infants who remained without atopic dermatitis	T-RFLP TGGE	Wang et al. (2008)
Faeces (3 time points)	16 Infants who developed allergy 31 Infants who remained non-allergic	qPCR	Sjögren et al. (2009)
<i>Autism</i>			
Faeces	13 Autistic children	Conventional culturing	Finegold et al. (2002)
Stomach contents	8 Non-autistic children	16S rRNA gene sequencing	
Small intestine contents			
Faeces	15 Autistic children 8 Non-autistic children	qPCR	Song et al. (2004)
Faeces	58 Autistic children 12 Non-autistic siblings 10 Non-autistic children	FISH	Parracho et al. (2005)
Faeces	33 Autistic children 7 Non-autistic siblings 8 Non-autistic children	454 FLX titanium sequencing (16S)	Finegold et al. (2010)
<i>Diabetes type 2</i>			
Faeces	16 Type 2 diabetic patients 12 Non-diabetic individuals	DGGE qPCR	Wu et al. (2010)
Faeces	18 Type 2 diabetic patients 18 Non-diabetic individuals	qPCR 454 FLX titanium sequencing (16S)	Larsen et al. (2010)
<i>Rheumatoid arthritis</i>			
Faeces	51 Patients with early rheumatoid arthritis 50 Patients with fibromyalgia	FISH/flow cytometry	Vaahntovu et al. (2008)

DGGE denaturing gradient gel electrophoresis, *FISH* fluorescence in situ hybridisation, *qPCR* quantitative polymerase chain reaction, *T-RFLP* terminal-restriction fragment length polymorphism, *TGGE* temperature gradient gel electrophoresis

microbiota composition and diversity to disease. Again, also for all these diseases the causality argument of the observed microbiota changes is unresolved. Ultimately,

causality and knowledge of the underlying mechanisms will be crucial for a full understanding of the role of the intestinal microbiota in the aetiology of specific diseases.

Modulation of the intestinal microbiota

Since it is known that the intestinal microbiota plays an important role in human health and disease, manipulation of these microorganisms by antibiotics, probiotics, prebiotics and synbiotics are attractive approaches to improve and maintain health (Gareau et al. 2010; Preidis and Versalovic 2009).

Antibiotics are widely used as antimicrobial agents to treat bacterial infections caused by pathogenic microorganisms. In general, however, antibiotics (even narrow-spectrum antibiotics) do not only affect pathogens, but also commensal intestinal microbial communities. This can result in dysbiosis of the intestinal microbiota, subsequently leading to intestinal problems, such as antibiotic-associated diarrhoea (AAD) (McFarland 1998). The antibiotic-induced disturbances in microbiota composition can be temporary, returning to its original composition within 2 months, but recently also medium and long-term disturbances in (specific) microbial communities have been described (Dethlefsen et al. 2008; Jernberg et al. 2007, 2010; Koning et al. 2010). An additional problem of the widespread antibiotic use, is the increased prevalence of antibiotic resistance resulting from the transfer of antibiotic resistance genes amongst microorganisms (Jernberg et al. 2010).

The intestinal microbiota can be modulated in a more biological manner by the use of probiotics. According to the definition formulated by the World Health Organisation (WHO) probiotics are ‘live microorganisms which, when administered in adequate amounts, confer a health benefit on the host’ (FAO/WHO 2002). Moreover, prebiotics are used to manipulate the microbiota composition in the GI tract. The definition of prebiotics is even more generic than the one of probiotics: ‘non-digestible food ingredients that, when consumed in sufficient amounts, selectively stimulate the growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host’ (Roberfroid et al. 2010). Mixtures of both probiotics and prebiotics are referred to as synbiotics.

The final part of this review will focus on probiotics as a way to modulate the intestinal microbiota. The opportunities for probiotic intervention in maintaining and restoring health are increasingly being acknowledged, and the field of probiotic research has grown significantly during the past few years (Gareau et al. 2010; Ng et al. 2009; Rijkers et al. 2010).

An introduction to probiotics

In line with the very generic definition of probiotics, many different microorganisms have been studied for their

potential use as a probiotic, in relation with a wide range of biological or clinical effects. Most of the microorganisms that have been studied are naturally present in the human GI tract. At this moment, the most commonly used probiotic microorganisms belong mainly to the bacterial genera *Lactobacillus* and *Bifidobacterium* (Boesten and De Vos 2008; Kleerebezem and Vaughan 2009). These two genera contain a large number of species and strains of which many are being used as probiotic strains. In addition to these genera, the probiotic market contains members from some additional lactic acid bacterial genera, such as *Streptococcus* and *Enterococcus*, and members from the genera *Bacillus* and *Propionibacterium*. Furthermore, some gram-negative bacteria (e.g. *E. coli* Nissle 1917) and yeast (e.g. *Saccharomyces*) are being used as probiotic microorganisms (Gareau et al. 2010; Holzapfel et al. 1998; Iannitti and Palmieri 2010).

Numerous health-beneficial effects have been attributed to probiotic microorganisms (Iannitti and Palmieri 2010; Ng et al. 2009). In general, these health benefits can be categorised into three levels of probiotic action (Rijkers et al. 2010). First of all, probiotic microorganisms can act directly within the GI tract (level 1), for example by direct interaction with the intestinal microbiota or by enzymatic activities. Secondly, they can interact directly with the intestinal mucus layer and epithelium (level 2), thereby influencing the intestinal barrier function and the mucosal immune system. Thirdly, probiotics can have effects outside the GI tract (level 3), for example on the systemic immune system and other organs, such as the liver and the brain. Although in vivo data are emerging, most of the mechanistic studies with probiotic microorganisms have been performed in vitro, ex vivo or with the aid of animal models. The in vitro activity of a given probiotic does not necessarily correlate with the efficacy of the intended clinical in vivo. In addition, it is important to note that each probiotic strain has its own specific properties. The health benefits that can be attributed to one probiotic strain cannot be extrapolated to other probiotic strains or mixtures of strains. Even closely related microbial strains of the same species may have different physiological effects (Medina et al. 2007; Meijerink et al. 2010; López et al. 2010; Snel et al. 2010; Van Hemert et al. 2010; Vissers et al. 2010, 2011).

Nowadays, multistrain or multispecies probiotic mixtures, which contain more than one probiotic strain, are becoming increasingly popular (Chapman et al. 2011; Timmerman et al. 2004). Compared with single strain probiotics, probiotic mixtures have the possible advantage that the properties of single strains may have additive or even synergistic effects when put in a mixture together with other probiotics strains, which can result in higher efficacy. Another potential advantage of probiotic mixtures

may be that compared with a single strain probiotic a wider range of health benefits could be accomplished. In contrast, mixing of probiotic strains might also result in reduced efficacy since individual strains may have opposite effects or even inhibit each other. There are, however, a limited number of *in vivo* studies available that compare the effects of a probiotic mixture with those of the individual strains (Chapman et al. 2011). This means that the evidence for the hypothesis that probiotic mixtures are more effective than the individual strains is still limited.

The influence of probiotics on the composition and diversity of the intestinal microbiota

Modulation of the intestinal microbiota (part of level 1 probiotic action) is one of the potential health-beneficial effects of probiotics. The mechanisms by which probiotic microorganisms are able to modify the intestinal microbiota include reduction of luminal pH, competition for nutrients, secretion of anti-microbial compounds (bacteriocins), prevention of bacterial adhesion and evasion of epithelial cells, and induction of the production of anti-microbial compounds (defensins) by the host (Fooks and Gibson 2002; Ng et al. 2009). By these mechanisms, probiotics can not only potentially modulate the intestinal microbiota composition, but also prevent pathogenic bacterial overgrowth.

Up to this date, many studies have been performed that examine the effects of probiotics on the composition and diversity of the intestinal microbiota, both in diseased and healthy individuals. For a given disease, the desired outcome of probiotic intervention is the modulation of the intestinal microbiota in such a way that a healthy microbiota composition is achieved. However, also other parameters have been addressed such as stabilisation of the microbiota as in the case of IBS and a multispecies probiotic, determined with the use of a phylogenetic microarray (Kajander et al. 2008). The interpretation of the effects of probiotics on the intestinal microbiota composition in healthy individuals are, however, more difficult to interpret (Table 7). Those studies do provide information on the effects of probiotics on the intestinal microbiota without a potential bias caused by disease effects. However, this does not imply that in a diseased situation these probiotic products will have the same influence on the intestinal microbiota.

Until recently, in most of the probiotic studies conventional culture-based methods have been used to study the influence of probiotics on the intestinal microbiota. However, since a few years culture-independent methods are now also being applied in probiotic research (Table 7). In general, demonstrating the colonisation of the supplemented probiotic microorganism(s) has been the primary

aim of most studies in healthy individuals. In most cases, a transient colonisation of the probiotic microorganism(s) has been observed. It is still questionable, however, whether probiotic strains would need to colonise in order to be effective or whether transient presence would also suffice to exert health-beneficial effects.

The probiotic studies performed in humans have almost exclusively examined the effect of probiotic administration on the composition of the faecal microbiota, whereas other niches of the GI tract have hardly been studied thus far (Table 7). As already indicated, even major local changes in microbiota composition in specific niches of the GI tract might not be reflected in the faeces. This means that there is still a major gap in knowledge on the influence of probiotic microorganisms on the intestinal microbiota. In addition, the influence of probiotic microorganisms on mucosa-associated intestinal microbiota is also not well studied. However, these interactions are possibly of key importance in relation to disease pathogenesis, since mucosa-associated microorganisms are in more close contact with the intestinal barrier and immune system. One of the few examples of a study on the *in vivo* effects of probiotics on the human host is a recent study by Van Baarlen et al. (2009). The authors examined the influence of a probiotic microorganism on human duodenal mucosal gene expression and they showed that changes in gene expression patterns, especially in the $\text{NF-}\kappa\text{B}$ dependent pathways, induced by *Lactobacillus plantarum* WCFS1 could be linked to the establishment of immunotolerance in human adults.

In contrast with most human probiotic studies, animal studies have focused on the spatial influence of probiotics on the intestinal microbiota (Table 7). However, to which extent these results reflect the human situation has to be determined. Administration of a given probiotic strain will result in the (temporarily) increase of that strain the GI tract, but may also change the overall composition of the intestinal microbiota. Indeed, the results of relevant experiments performed thus far demonstrate that probiotic-induced changes in microbiota composition are not restricted to the administered species. Which probiotic microorganisms are able to influence the relative abundance of which specific intestinal microorganisms are questions that are currently under study. It should be realised, however, that a change in composition or diversity of the intestinal microbiota by probiotic intervention is not a health benefit by itself.

As discussed previously, dysbiosis of the intestinal microbiota has been associated with a growing number of (intestinal) diseases. Since modulation of the composition of intestinal microbiota by probiotics was demonstrated to be possible, probiotic intervention has the potential to counterbalance intestinal dysbiosis and thus restore health.

Table 7 Details of studies performed to examine the effects of probiotic intervention on intestinal microbiota composition of healthy subjects determined with culture-independent methods

Population	Study groups (based on treatment)	Study material	Analytical methods	Key findings	References
<i>Animals</i>					
Healthy rats (<i>n</i> = 30)	Probiotic: <i>B. lactis</i> B1 and <i>S. thermophilus</i> Prebiotic: FOS Placebo: only carrier material	Caecum (tissue and contents)	Conventional culturing DGGE	Both prebiotic and probiotic group: ↓ <i>Clostridia</i> ↓ <i>Bacteroides</i> ↓ total anaerobes Prebiotic-treated group: ↓ coliforms ↑ <i>Bifidobacterium</i> Probiotic-treated group: ↑ diversity ↑ coliforms	Montesi et al. (2005)
Healthy mice (<i>n</i> = 16)	Probiotic: <i>L. casei</i> Probiotic: <i>L. plantarum</i> Probiotic: mixture of <i>L.</i> <i>casei</i> and <i>L. plantarum</i> Control: no treatment	Faeces Intestinal tissue	DGGE T-RFLP Clone-library sequencing	Mixture-treated group: No significant effect on dominant microbiota composition Shifts in the diversity of <i>Lactobacillus</i> species	Fuentes et al. (2008)
Healthy fish (red tilapia) (<i>n</i> = 12)	Probiotic: diet containing <i>Pediococcus acidilactici</i> Placebo: normal diet	Intestinal contents	Conventional culturing DGGE	Probiotic-treated group: ↓ Species richness and diversity Transiently colonization by <i>P. acidilactici</i>	Ferguson et al. (2010)
<i>Humans</i>					
Healthy adults (<i>n</i> = 10)	Probiotic: milk powder containing <i>L. rhamnosus</i> DR20	Faeces	Conventional culturing FISH DGGE	Probiotic-treated group: No significant effect on dominant microbiota composition	Tannock et al. (2000)
Healthy adults (<i>n</i> = 30)	Probiotic: <i>B. animalis</i> subsp. <i>lactis</i> Bb-12 Prebiotic: GOS Synbiotic: GOS and <i>B. animalis</i> subsp. <i>lactis</i> Bb-12	Faeces	DGGE	All groups: No qualitative changes in faecal <i>Bifidobacterium</i> communities Probiotic/synbiotic-treated groups: Transiently colonization by <i>B. animalis</i> subsp. <i>lactis</i> Bb-12	Satokari et al. (2001)
Healthy children (<i>n</i> = 26)	Probiotic: Yoghurt containing <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>S. thermophilus</i> and <i>L. paracasei</i> A Placebo: pasteurised yoghurt	Faeces	Conventional culturing RAPD-PCR DGGE	Probiotic-treated group: No significant effect on dominant microbiota composition GI survival and transiently colonization by <i>L. paracasei</i> A	Marzotto et al. (2006)
Preterm infants (<i>n</i> = 69)	Probiotic: <i>B. animalis</i> subsp. <i>lactis</i> Bb-12 Placebo: only carrier material	Faeces	Conventional culturing FISH	Probiotic-treated group: ↑ <i>Bifidobacterium</i>	Mohan et al. (2006)

Table 7 continued

Population	Study groups (based on treatment)	Study material	Analytical methods	Key findings	References
Healthy adults (<i>n</i> = 12)	Probiotic: yoghurt containing <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>S. thermophilus</i> and <i>B. animalis</i> DN-173 010 Probiotic: <i>B. animalis</i> DN-173 010 (lyophilised)	Faeces	Conventional culturing Colony immunoblotting DGGE FISH	Both probiotic-treated groups: No significant effect on dominant microbiota composition GI survival and transiently colonization by <i>B. animalis</i>	Rochet et al. (2008)
Healthy adults (<i>n</i> = 30)	Prebiotic: lactulose Probiotic: <i>S. boulardii</i> Synbiotic: lactulose and <i>S. boulardii</i> Placebo: maltodextrin	Faeces	DGGE Group-specific qPCR	Prebiotic-treated group: ↑ <i>B. adolescentis</i> ↑ <i>Bifidobacterium</i> Probiotic/synbiotic-treated group: No changes	Vanhoutte et al. (2006)
Healthy elderly (<i>n</i> = 55)	Probiotic: fermented oat drink containing <i>B. longum</i> 46 and <i>B. longum</i> 2C Probiotic: fermented oat drink containing <i>B. animalis</i> subsp. <i>lactis</i> Bb-12 Placebo: only fermented oat drink	Faeces	Species-specific qPCR	Probiotic-treated group (<i>B. longum</i>): ↑ <i>B. adolescentis</i> ↑ <i>B. catenulatum</i> Probiotic-treated group (<i>B. animalis</i>): ↑ <i>B. animalis</i>	Ouwehand et al. (2008)
Healthy adults (<i>n</i> = 14)	Probiotic: encapsulated <i>L. rhamnosus</i> R11 and <i>L. acidophilus</i> R52	Faeces	Conventional culturing qPCR	No significant effect on dominant microbiota composition GI survival and transiently colonization by <i>L. rhamnosus</i>	Firmesse et al. (2008)
Healthy adults (<i>n</i> = 79)	Probiotic: yoghurt containing <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> and <i>S.</i> <i>thermophilus</i> Placebo: pasteurised yoghurt Control: no yoghurt	Faeces	DGGE qPCR	Probiotic-treated group: ↑ lactic acid bacteria ↑ <i>C. perfringens</i> Yoghurt-receiving groups: ↓ <i>Bacteroides</i> group	García-Albiach et al. (2008)
Healthy adults on antibiotic treatment (<i>n</i> = 40)	Probiotic: <i>B. animalis</i> subsp. <i>lactis</i> BI-04, <i>B. animalis</i> subsp. <i>lactis</i> Bi-07, <i>L. acidophilus</i> NCFM, <i>L. paracasei</i> Lpc-37, <i>B. bifidum</i> Bb-02 and maltodextran Placebo: only maltodextran	Faeces	Conventional culturing T-RFLP	Probiotic-treated group: A more rapid return to pre-antibiotic microbiota composition ↑ <i>Enterobacteriaceae</i> ↑ <i>Bifidobacterium</i>	Engelbrektsen et al. (2009)
Healthy elderly (<i>n</i> = 66)	Probiotic: fermented oat drink containing <i>B. longum</i> 46 and <i>B. longum</i> 2C Placebo: non-fermented oat drink	Faeces	Conventional culturing Species-specific qPCR	Probiotic-treated group: Significant change in <i>Bifidobacterium</i> communities (↑ <i>B. catenulatum</i> , <i>B. bifidum</i> and <i>B. breve</i>)	Lahtinen et al. (2009)

DGGE denaturing gradient gel electrophoresis, FISH fluorescence in situ hybridisation, qPCR quantitative polymerase chain reaction, RAPD-PCR random amplification of polymorphic DNA polymerase chain reaction, T-RFLP terminal-restriction fragment length polymorphism

The effectiveness of probiotic intervention has been studied in a number of human diseases, including IBD (CD, UC and pouchitis), IBS, constipation, diarrhoea (including AAD), colon cancer, cardiovascular disease, NEC, allergic diseases, obesity and metabolic disorders and these have been the subject of systematic reviews as well as Cochrane reviews (Gareau et al. 2010; Iannitti and Palmieri 2010; Pham et al. 2008; Sanz et al. 2010; Weichselbaum 2010). With the possible exception of NEC and pouchitis, variable clinical effects are found. One, and probably the most important reason for the variable clinical effects is the variation in probiotic species and strains that are being used. On top of that, there is a lack of standardised methods for the study of the intestinal microbiota (e.g. sample collection, sample storage and analysis methods), which makes it almost impossible to directly compare findings from different groups. Apart from the large variety of probiotic species and strains, also different dosages of probiotic microorganisms are used, or combinations of probiotic species and strains, or prebiotic supplements can be added. In addition, the populations of interest can be relatively heterogeneous since health and disease are not always well defined. At the same time, host-dependent factors (e.g. host genotype) may have an influence on the effectiveness of intestinal microbiota modulation by probiotics. Finally, most clinical studies have included only a small number of patients and used short-term intervention periods. All of this, in combination with the fact that the intestinal microbiota composition is diverse and maybe even unique for each individual, makes it problematic to observe general changes in microbiota composition as result of probiotic intervention.

In the early days of probiotic research, it was thought that decreased intestinal microbial diversity could be a direct cause of gastrointestinal disease. In such a concept, probiotic intervention should be aimed at increasing this diversity, which would be sufficient to resolve the clinical problem. For some diseases such as IBD, there is indeed evidence for a decreased diversity (Dicksved et al. 2008; Nishikawa et al. 2009; Qin et al. 2010). By contrast, a recent culture-independent study shows a higher richness and diversity of bacteria in the faeces of autistic individuals compared with healthy controls (Finegold et al. 2010). Nowadays, it is recognised that the interaction between intestinal microbiota and the host is more complex than just a high or low microbial diversity. Thus, no general statements can be made on the role of microbial diversity in health and disease, since different microbe-host interactions are involved in the pathophysiology of different diseases. Knowledge of the molecular and physiological mechanisms behind specific diseases and aberrations that are associated with microbial dysbiosis will contribute to

the development of tailor-made probiotics designed for specific interventions.

Application of high-throughput molecular approaches in probiotic research

New insights in the potential effect of probiotic intervention on the intestinal microbiota can be obtained by application of high-throughput molecular approaches in probiotic research. An example is provided by a study in which the effectiveness of daily probiotic supplementation of *Lactobacillus rhamnosus* GG (LGG) on preventing the development of early markers of asthma in a human clinical study was examined (Cabana et al. 2007). The probiotic bacterium LGG is one of the most widely used probiotic microorganisms and has been used in a large number of clinical trials. An explanation for its probiotic properties has recently been provided by its genomic characterisation revealing the presence of mucus binding pili in LGG that are assumed to interact with the host (Kankainen et al. 2009). A phylogenetic microarray analysis was used to study the effect of LGG abundance on the bacterial community structure of stool samples of 6-month-old infants (Cox et al. 2010). Since the researchers were blinded to the treatment of the infants (probiotic or placebo), the effect of LGG administration on LGG abundance and intestinal microbiota composition could not be examined. However, cluster analysis of the microarray data demonstrated that LGG abundance was associated with a distinct community composition. Communities with high relative abundance of LGG showed an increased relative abundance of a large number of bacterial taxa and the majority of these taxa were phylogenetically clustered. In addition, there was a significant difference in evenness of the intestinal microbiota between samples containing a low or high abundance of LGG. It was hypothesised that a possible mechanism of the probiotic action of LGG is the stimulation of a stable, even and functionally redundant microbiota and facilitating the colonisation by other beneficial microorganisms (Kankainen et al. 2009). Whether the ability of pili of LGG to bind to intestinal mucosal surfaces is important in this respect remains to be determined.

Recently, high-throughput metagenomic sequencing was used to relate the effect of probiotic intervention to microbiota composition (Veiga et al. 2010). It was demonstrated that consumption of a fermented milk product supplemented with *Bifidobacterium animalis* subsp. *lactis* (BFMP) induced some specific metabolic shifts in an ulcerative colitis mouse model. In addition, it was shown that the immune status of the mice had an effect on the shifts in the composition of the intestinal microbiota.

Moreover, subsets of mice could be identified based on microbiota composition that clustered together corresponding to effectiveness of the BFMP treatment. These results reinforce the notion that the composition of the endogenous intestinal microbiota plays an important role in the host response to the probiotic intervention, thereby influencing the effectiveness of probiotic intervention. In this study, it was also observed that BFMP consumption resulted in a metabolic shift; a decreased caecal pH and alteration in short-chain fatty acid profiles. It must be noted, however, that these beneficial effects cannot be directly linked to the activity of this specific *Bifidobacterium* strain since a non-fermented milk product was used as control product. Overall, the data support the hypothesis that probiotics are not only able to influence the composition, but also the metabolic activity of the intestinal microbiota (De Preter et al. 2010). Both of these effects need to be studied separately to get a complete picture of the influence of probiotic intervention on the intestinal microbiota. This is also emphasised in a recent study in which the effect of a specific synbiotic product on the intestinal microbiota was examined (Vitali et al. 2010). This study showed no influence on the composition of dominant faecal microbiota, but significant changes in faecal metabolic profiles were observed. These results suggest that synbiotic intervention is able to affect the metabolic activity of the intestinal microbiota while maintaining microbiota composition with respect to its predominant components.

Conclusions

Knowledge on the composition and diversity of a healthy microbiota and on how changes in the intestinal microbiota lead to or are associated with disease, is far from complete. More research is needed to examine the species and strain diversity in the GI tract, the diversity of microbial genes (microbiome) in the GI tract and the activity of these genes (mRNA, protein and metabolite production). For future probiotic research it is important to determine the level of compositional and functional microbial dysbiosis in relevant target populations and identify potential members of the healthy microbiota to counteract the dysbiosis. Understanding the molecular mechanisms of action attributed to commensal and pathogenic bacteria will contribute to better designed probiotic products. In the future, this knowledge can be applied in the development of tailor-made probiotics designed for clearly characterised target populations.

Acknowledgments This work was partly funded by Agentschap NL, project FND-07013.

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