

16 Ecological Interactions of Bacteria in the Human Gut

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16.1 Introduction

The colon or large intestine is one of the most important organs of the human body (Macfarlane and Cummings, 1991). Moreover, its inhabitants, the colon microbiota, are the key elements of the human digestive ecosystem. The vast complexity of the human large-intestinal microbiota has inspired researchers to consider it as an organ itself, located inside the colon and acquired postnatally (Bäckhed et al., 2005; Zocco et al., 2007). From a physiologist's point of view, this image of the colon microbiota is relevant: like an organ, it is composed of different cell lineages that communicate with both one another and the host; it consumes, stores, and redistributes energy; it mediates physiologically important chemical transformations; and it is able to maintain and repair itself through self-replication (Bäckhed et al., 2005). As a microbial organ, the human colon community does not only broaden the digestive abilities of the host (Gill et al., 2006), but also influences body processes far beyond digestion (Roberfroid, 2005b; Turnbaugh et al., 2007).

From a microbiologist's point of view, the simplified perception of the microbial colon community as a functional entity narrows down colon ecosystem research toward a 'black box' approach, not only with respect to the identification of its inhabitants, but also concerning the numerous metabolic activities and interactions that take place in the large intestine (Pryde et al., 2002). As the exact composition of the colon microbiota remains at present largely unexplored, input/output-based studies might appear manageable tools to investigate a terrifying complexity. However, even though such studies would generate comprehensive results, they inherently neglect the sometimes subtle fluctuations in composition and metabolic activity within the microbial colon community. Such changes – with a possible impact on the host's health – can only be monitored after thorough dissection of the colon microbiota and subsequent identification

of the dominant microbial clusters and their interactions (Flint et al., 2007). The latter will eventually lead toward a global, ecological understanding of the colon ecosystem.

During the last few years, the implementation of culture-independent molecular techniques in the field of gut research has revealed the presence of an unsuspected microbial diversity in the human colon (Eckburg et al., 2005; Frank et al., 2007; Li et al., 2008). Further exploration of this microbial wealth is a necessary step toward a better understanding of the relationship between the human host and its symbionts. However, defining a complex ecosystem, such as the human large intestine, does not end with the construction of a catalogue of its members, but also implies the determination of the habitat (ecological or environmental area inhabited) and niche (relational position within the ecosystem) of each (cluster of) inhabitant(s) and its functional role(s) (Ley et al., 2006). This functional characterization of large bacterial groups within the colon ecosystem is probably one of the greatest challenges for microbiologists in the years to come (Turnbaugh et al., 2007).

16.2 Defining the Colon Ecosystem

The human colon represents one of the most complex microbial ecosystems known to men (Zoetendal et al., 2006). The concept of an ecosystem as the basic unit in ecology was first proposed by Arthur George Tansley in 1935 (Trudgill, 2007). It was defined as a natural unit consisting of all plants, animals, and micro-organisms (biotic factors) in an area functioning together with all of the non-living physical (abiotic) factors of that environment (Evans, 1956; Tansley, 1935; Townsend et al., 2003). An ecosystem is by definition an artificial subunit of an environment: a mental construct imposed on a complex reality for study purposes solely (Tansley, 1935). Ecosystem boundaries are mental boundaries, as all ecosystems are not only part of larger ones, but also overlap, interlock, and interact with one another (Tansley, 1935; Townsend et al., 2003). The human colon ecosystem, characterized by a constant in- and outflux of nutrients, microorganisms, and end-products of colon fermentation, makes no exception to this rule.

From a broad ecological point of view, the human body can be considered as a super-organism composed of an amalgam of both microbial and human cells, depending on each other for their survival (Lederberg, 2000). The relationship between the host and the microbial populations inhabiting its colon is often described as commensal (one partner benefits while the other seems unaffected)

as opposed to mutualistic (both partners derive benefit) (Bäckhed et al., 2005; Hooper and Gordon, 2001; Ley et al., 2006). However, it has been suggested that the use of this denomination only reflects a current lack of knowledge concerning the exact nature of host-microbiota interactions (Bäckhed et al., 2005).

The activities of the colon microbiota have a major impact upon nutrition and health of the host via the supply of nutrients, conversions of metabolites, and interactions with host cells (Flint et al., 2007). Moreover, it has been argued that the mere stability of the colon ecosystem, as a result of partially host-driven selection, contributes to the health and well-being of an individual (Ley et al., 2006).

16.2.1 The Human Colon Environment

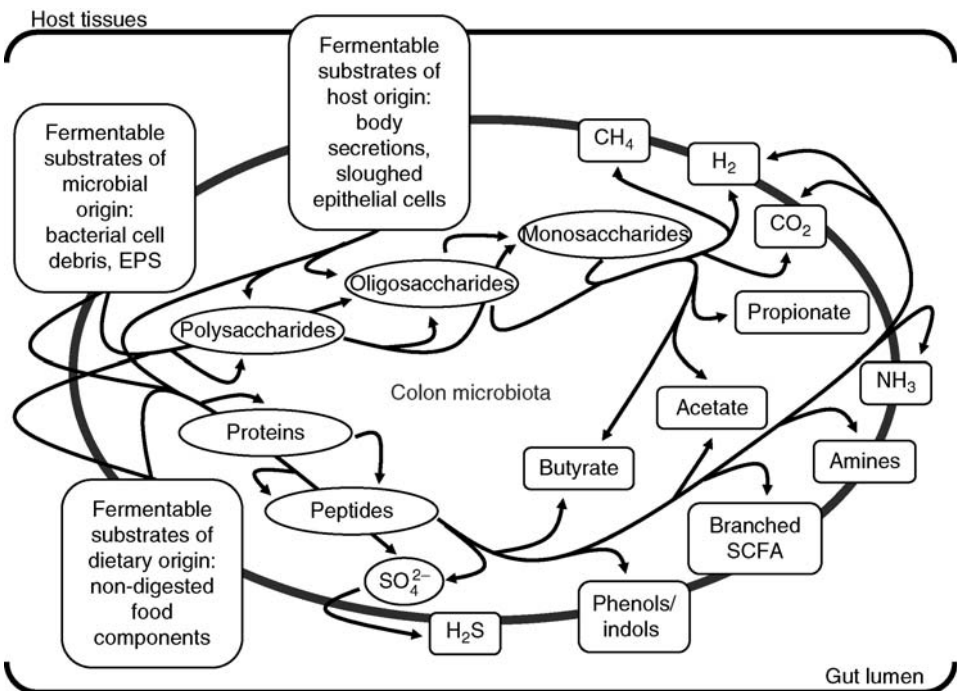
The human colon can be pictured as an unbranched tube into which nutrients enter at one side and feces are excreted at the other (Macfarlane and Cummings, 1991). It has an average length of 1.5 m and an undisected surface of approximately 1.3 m². The large intestine contains about 220 g of wet contents, including an estimated total bacterial mass of 90 g. Daily, it receives around one to two liters of chyme – a semi-fluid mass composed of (partially) undigested food ingredients ($\pm 95 \text{ g day}^{-1}$), body excretions, and bacterial as well as human cells – which is subsequently reduced to a final volume of approximately 0.2 l semi-solid feces as a result of absorption of fluids (Macfarlane and Cummings, 1991; Roberfroid, 2005b). Transit of contents is relatively slow, with an average transit time of 60 h in people living on a Western-type diet, whereby gut transit always slows down from the proximal colon toward the rectum (Macfarlane and Cummings, 1991). However, the constant flow of nutrients through the gut ecosystem forces colon bacteria to avoid washout by either maintaining a sufficient reproduction rate or attaching to or colonizing host tissues (Flint et al., 2007).

Roughly, the colon can be divided into three different regions with respect to nutrient availability and bacterial activity, namely the proximal or ascending colon, the transversal colon, and the distal or descending colon (Macfarlane and Cummings, 1991). Food residues and nutrients (carbohydrates, proteins, etc) not absorbed in the small intestine primarily arrive in the ascending colon, where they can be fermented by the resident microbiota. High carbohydrate availability results in high growth rates, fast bacterial turnover, and a relatively low luminal pH of 5.4–5.9. The gradual carbohydrate and moisture depletion toward the end of the descendal colon is reflected in lower growth rates, slower bacterial turnover, and a corresponding luminal pH of 6.6–6.9, as compared to the proximal colon.

Whereas carbohydrate fermentation occurs mainly in the proximal colon, protein degradation increases progressively toward the end of the distal part of the colon (Macfarlane et al., 1992). End-products of carbohydrate fermentation include short-chain fatty acids (SCFA), such as acetate, propionate and butyrate, and gases (see Section Dietary Carbohydrate Metabolism in the Human Colon: Metabolic Cross-Feeding), while protein fermentation also generates branched SCFA, ammonia, amines, phenols, and indols (▶ [Figure 16.1](#)) (Roberfroid, 2005a). The transversal colon is characterized by intermediate conditions with lower bacterial growth rates as compared to the proximal colon and a luminal pH at an intermediate value of approximately 6.2.

16.2.2 Spatial Heterogeneity

The human colon ecosystem represents in terms of cell density the most successful microbial ecosystem presently described (Ley et al., 2006). The human intestinal



■ **Figure 16.1**

Overview of the main routes of carbohydrate and protein fermentation in the human colon.

tract is packed with up to 100 trillion microorganisms, reaching cell densities of up to 10^{12} cells ml^{-1} toward the end of the gut – the highest recorded number for any microbial habitat up to now (Whitman et al., 1998). The human microbiome (the collective genome of the microbiota) is thought to contain more than 100 times the total number of human genes, making the human being more prokaryote than eukaryote (Bäckhed et al., 2005; Gill et al., 2006). The key of the success of the human colon as a microbial habitat lies in the fact that it represents a nutrient-rich and at the same time spatially heterogeneous environment (Flint et al., 2007). While the abundance of nutrients is indispensable to comply with the carbon and energy requirements of the enormous numbers of residing microorganisms, the heterogeneity of the environment assures the availability of a multitude of ecological niches (Flint, 2006; Ley et al., 2006).

The spatial heterogeneity of the colon originates from a variety of factors including host characteristics (e.g., genotype, immune status) and diet composition (e.g., degree of consumption of meat and vegetables/fruits, level of carbohydrate and protein uptake, consumption of pre- and probiotics). Moreover, niche diversity is enhanced by the development of a complex network of often nutrition-based microbial interactions within the colon microbiota itself, with cooperation and competition as the main driving forces (Flint et al., 2007; Flint, 2006). Cooperative interspecies interactions can occur during hydrolysis of complex carbohydrates and sequential utilization of fermentation and partial breakdown products, as well as by the exchange of growth factors (Dethlefsen et al., 2006; Flint et al., 2007). Competition for resources and adhesion sites to the colon are considered the main limiting factors for microbial growth, combined with other bacterial interference mechanisms such as the production of toxic metabolites and specific antimicrobial compounds such as bacteriocins (Dethlefsen et al., 2006; Makras and De Vuyst, 2006; Makras et al., 2004). In this context, the abundant and diverse presence of phages should be mentioned as a possible interfering component, which may contribute to the diversity of the colon's microbial genetic landscape (Breitbart et al., 2003).

Besides competitive and cooperative interactions, colonization history is considered a microbial factor adding to niche diversity (Curtis and Sloan, 2004; Tilman, 2004). By colonizing a pre-existing microbial niche, microorganisms influence their direct surroundings and locally alter the colon environment. In a way, the assembly of the colon microbiota can be thought of as being partially recursive, creating and responding to its own selective pressure (Day et al., 2003). The influence of colonization history contributes to interindividual differences in microbiota composition (see Section Defining the Colon Ecosystem: Microbial

Diversity). Moreover, it enhances and extends the consequences of historical contingencies such as antibiotic interventions (Dethlefsen et al., 2006). In this respect, the colon microbiota does not necessarily represent a climax population: internal dynamics can assure the stable persistence of an out-of-equilibrium situation (Sarr et al., 2005).

As a consequence of the interplay between host and dietary factors, combined with the effects of microbial interactions, the human colon environment is populated with extensively adapted species, displaying a high level of interdependence – often translated in complex growth requirements and possible obligate interactions such as syntrophy (Flint et al., 2007; Ley et al., 2006). The latter is not only reflected by the difficulties encountered when attempting to isolate individual members of the resident microbiota (Ducluzeau, 1989; Duncan et al., 2007b), but it also complicates the prediction of the human colon community responses to external factors – even when isolated populations respond deterministically (Dethlefsen et al., 2006; Flint et al., 2007).

16.2.3 Microbial Diversity

For many decades, the use of culture-dependent microbiological investigation techniques, involving selective plating and incubation, and subsequent identification of bacterial isolates, has dominated gut diversity research (Macfarlane and Macfarlane, 2004; Tannock, 1999). Although the application of such traditional microbiological methods has contributed substantially to the current understanding of the composition of the colon ecosystem, cultivation biases gave rise to some persistent myths concerning the dominating genera. Due to the lack of total anaerobic culturing techniques and the use of cultivation media that do not meet the complex nutritional demands of many colon inhabitants, the presence of relatively easily culturable microorganisms with a high degree of oxygen tolerance has been overestimated in the past (Walter, 2008). Such microorganisms include lactobacilli, clostridia, enterococci, and, on the species level, *Escherichia coli*, as was later revealed by the introduction of culture-independent molecular techniques in the world of gut microbiology (Eckburg et al., 2005; Frank et al., 2007; Walter, 2008). At this moment, up to 80% of the bacterial species inhabiting the human colon is considered unculturable using presently available methodologies (Blaut et al., 2002; Duncan et al., 2007b; Hold et al., 2002). However, it has been suggested that this percentage mainly reflects the limited recent efforts on cultivation rather than an inherent culturability (Flint et al., 2007).

Nowadays, the most comprehensive and least biased enumerations of microbial diversity come from sequencing of 16S rRNA genes, obtained directly from DNA extracted from colon samples, using PCR primers targeted to broad phylogenetic groups (Ley et al., 2006). The main conclusion of the first attempts to implement such techniques on a large scale basis is the fact that microbial colon diversity has been largely underestimated throughout the years (Eckburg et al., 2005; Frank et al., 2007). Diversity at the strain level is overwhelming: estimations vary between 500 and 1,000 species, representing over 7,000 strains (Hooper and Gordon, 2001; Xu et al., 2007). However, despite the large progress that has been made during recent years, current knowledge of the composition of the human colon microbiota remains partial, fragmented, and undetailed.

Notwithstanding the huge diversity reported at strain level, the human colon appears to be a remarkably selective environment. Recent 16S rRNA gene sequencing-based surveys of the distal gut and fecal microbiota of adult individuals revealed that the colon ecosystem is dominated by only four of the 55 bacterial divisions (deep evolutionary lineages, sometimes referred to as ‘phyla’) described up to date (Bäckhed et al., 2005; Eckburg et al., 2005; Frank et al., 2007). Together, species belonging to the divisions *Firmicutes* (64% – mostly belonging to the *Clostridia* class, including the genera *Eubacterium*, *Faecalibacterium*, *Roseburia*, and *Lactobacillus* – the latter representing a minor group), *Bacteroidetes* (23% – mainly *Bacteroides* spp., with *Bacteroides thetaiotaomicron* as a common representative), *Proteobacteria* (8% – among which *Enterobacteriaceae* and sulfate-reducing colon bacteria), and *Actinobacteria* (3% – including *Bifidobacterium* spp.) make up more than 98% of the bacterial colon population (▶ [Table 16.1](#)) (Eckburg et al., 2005; Frank et al., 2007). Other bacterial divisions less abundantly encountered include *Cyanobacteria*, *Fusobacteria*, *Spirochaetes*, and *Verrucomicrobia* (Bäckhed et al., 2005; Eckburg et al., 2005; Frank et al., 2007; Ley et al., 2006). Of the 13 archaeal divisions known to date, *Methanobrevibacter smithii* seems to be the only common resident of the human large intestine (Bäckhed et al., 2005; Eckburg et al., 2005), although some studies indicate a greater diversity (Scanlan et al., 2008). Reports on the presence of fungi are scarce (Scupham et al., 2006).

The reasons for the selectivity of the colon environment are to be found in the strict requirements for the colon microbial community membership. The requisites needed include an arsenal of enzymes to utilize available nutrients; cell-surface molecular paraphernalia to attach to a suited habitat, evade bacteriophages, and appease the human immune system; a genetic flexibility toward mutation and adaptation; the ability to avoid washout through rapid proliferation; and the stress resistance needed to be able to cross a toxic, dry, and aerobic environment when

■ **Table 16.1**

Niches of some prominent microbial groups in the human colon (Dethlefsen et al., 2006; Duncan et al., 2007b; Flint, 2006; Macfarlane and Cummings, 1991)

<p><i>Firmicutes</i> – Clostridial cluster IV. Contains <i>Faecalibacterium prausnitzii</i>, an abundant fermenter of starch and inulin-type fructans with an absolute requirement for acetate. Produces butyrate and lactate. Other Cluster IV bacteria have been reported to degrade a range of carbohydrates, including starch, fucose, cellulose (<i>Ruminococcus</i> spp. – in combination with methane producers), and other complex plant cell wall materials. Metabolites reported are butyrate, lactate, and acetate.</p>
<p><i>Firmicutes</i> – Clostridial cluster IX. Poorly characterized bacterial group. Includes species belonging to the genera <i>Megasphaera</i> and <i>Veillonella</i>, among others. Predominantly saccharolytic. Lactate consumption by <i>Megasphaera elsdenii</i> has been reported. Production of butyrate and propionate.</p>
<p><i>Firmicutes</i> – Clostridial cluster XIVa. Broad bacterial cluster including species belonging to the genera <i>Anaerostipes</i>, <i>Clostridium</i>, <i>Coprococcus</i>, <i>Eubacterium</i>, <i>Roseburia</i>, and <i>Ruminococcus</i>. Mainly saccharolytic, growth reported on fucose, starch, xylan, and inulin-type fructans. Lactate conversion by <i>Anaerostipes caccae</i> and <i>Eubacterium hallii</i>. Butyrate production with (<i>Anaerostipes</i>, <i>Roseburia</i>, <i>Eubacterium</i>) or without (<i>Clostridium</i>, <i>Coprococcus</i>) acetate requirement. Other reported metabolites are ethanol, lactate, succinate, and propionate. Acetogenesis demonstrated for <i>Ruminococcus hydrogenotrophicus</i>.</p>
<p><i>Firmicutes</i> – scattered. Predominant proteolytic and amino acid-fermenting colon bacteria, mostly belonging to the genera <i>Peptococcus</i>, <i>Peptostreptococcus</i>, and <i>Clostridium</i>. Phylogenetically scattered group.</p>
<p><i>Bacteroidetes</i>. Mainly composed of species belonging to the genus <i>Bacteroides</i>. Degradation of xylan, mucin, inulin-type fructans, and other poly- and oligosaccharides. Periplasmic starch degradation. Includes some proteolytic bacteria belonging to the genera <i>Cetobacterium</i> and <i>Alistipes</i>. Production of acetate, propionate, and succinate.</p>
<p><i>Actinobacteria</i>. Contains bacterial species belonging to the genera <i>Bifidobacterium</i> and <i>Atopobium</i>. The genus <i>Bifidobacterium</i> includes starch-, inulin-type fructan-, xylan-, and mucin-degrading species, producing lactate, acetate, formate, and ethanol. <i>Atopobium</i> mainly produce acetate, formate, and lactate.</p>
<p><i>Proteobacteria</i>. The δ-subdivision of the <i>Proteobacteria</i> phylum includes H₂-consuming, sulphate-reducing bacteria, belonging to the genera <i>Desulfovibrio</i> and <i>Desulfobulbus</i>. Other genera consume partially reduced fermentation products, such as lactate and ethanol, while reducing sulphate to sulfide.</p>
<p><i>Archaea</i>. <i>Methanobrevibacter smithii</i> is the predominant methanogen present in the human colon.</p>

passed on from one host to another (Ley et al., 2006). A shallow, widely radiated, fan-like phylogenetic architecture (wide diversity at strain level but far fewer intermediate and deep lineages) is a typical feature for communities that suffer extreme selection followed by a period of détente (Ley et al., 2006). Only limited groups of bacteria are genetically armed to be able to reach the human colon alive,

but once settled, they find to their disposition a wide range of habitats and niches allowing them to proliferate and – on a microcommunity level – to differentiate.

16.2.4 Ecosystem Stability

Microbial ecosystems are generally characterized by a remarkable degree of stability (Sonnenburg et al., 2004; Walter, 2008). The human colon ecosystem makes no exception to this rule: although the colon microbiota is dynamic and able to adapt to environmental challenges, it remains relatively stable over time. It is characterized by a remarkable resistance to chaotic blooms of subpopulations and resilience after disturbances caused by, for example, antibiotic therapy, stress, or drastic changes in diet (Bäckhed et al., 2005; Ley et al., 2006; Makras et al., 2004). The latter phenomenon, commonly referred to as colonization resistance, mainly finds its origin in the highly niche-specific nature of most colon inhabitants. As every ecological niche can only support one or a conjunction of a limited number of microorganisms, it becomes extremely difficult for other bacteria to claim their piece of the environmental colon cake (Ley et al., 2006; Makras et al., 2004).

A key element in the construction process of a stable gut ecosystem is the development of oral tolerance by an individual in the first few months after birth. Oral tolerance, or the specific suppression of cellular and humoral immune responses to an antigen by prior administration of this antigen through the oral route, prevents hypersensitive reactions toward food proteins and bacterial antigens present in the gut environment (Weiner, 2000). It leads toward an individual acceptance of a particular microbiota by the human immune system – a peaceful coexistence between the human super organism and its microbial gut residents – while unaccepted elements might cause responses.

Another factor contributing to the stability of a microbial ecosystem is the diversity displayed by a bacterial community. A huge degree of diversity is usually translated into a broad repertoire of stress responses, ensuring the stability of key system processes (Ley et al., 2006). In the colon, this stability is of supreme importance, as drastic changes can severely affect human health (Bäckhed et al., 2005). Host selection for a stable colon microbiota avoids the occurrence of keystone species – defined as species with a unique and central role in a bacterial community – and favors functional redundancy among the residing microorganisms (Ley et al., 2006). As the human colon microbiota is characterized by shallow levels of microbial diversity (Eckburg et al., 2005; Frank et al., 2007), the latter selectivity for functional redundancy is reflected as habitat specialization. Within

the same bacterial genus, different ecotypes have emerged: functional redundant species coexisting by expressing different degrees of substrate specificity and degradation efficiency (Bäckhed et al., 2005). This combination of both specialization and generalization does not only ensure the stability of the ecosystem as a whole, but also provides individual strains with the ability to respond to a variety of different stress factors (Bäckhed et al., 2005).

The stability of the colon ecosystem makes it possible to differentiate between autochthonous microorganisms, true residents of a particular niche within the colon ecosystem, and allochthonous microorganisms, 'hitchhiking' through the gut (Ley et al., 2006; Walter, 2008). Autochthonous microorganisms have developed long-term associations with their particular hosts. They form stable populations in particular regions of the human colon, although the composition of those bacterial communities can differ substantially from one host to the other (Eckburg et al., 2005; Frank et al., 2007). Whereas a core composed of the dominant phyla is thought to be present in every individual, fingerprints of the colon microbiota at species/strain level are individual-dependent (Ley et al., 2006; Turnbaugh et al., 2007). The allochthonous colon population is mainly composed of microorganisms originating from ingested food, water, or other components from the environment (Ley et al., 2006). Bacteria considered autochthonous in other body ecosystems, such as the oral cavity, also contribute to the allochthonous colon community (Walter, 2008; Xu and Gordon, 2003). Although the composition of this subgroup of colon inhabitants can differ greatly in time, all members share the transient character of their large-intestinal existence.

Although the human gastro-intestinal tract is germ-free at birth, an immediate vertical transfer of microorganisms occurs between the mother and her baby. It has been shown that babies acquire their initial colon microbiota from the vagina and feces of the mother and/or from the environment (Ley et al., 2006). Due to the stability of the colon ecosystem, kinship relations remain observable over time. Through childhood, the complexity of the colon microbiota increases gradually (Dethlefsen et al., 2006). This evolution toward a higher degree of complexity persists in adulthood, as the colon ecosystem is a particularly open environment and, therefore, highly susceptible to colonization and perturbation. Moreover, while the harsh conditions of microbiota transfer at birth favor cooperation and altruism among gut-colonizing species, the period of détente that follows offers possibilities to cheaters, colon inhabitants that benefit from the residing community without contributing to it themselves. However, such cheaters remain a minority, limiting the probability that they are immediately transferred to a new-born (Ley et al., 2006).

16.2.5 Spatial Organization of Microbial Communities

Human feces are without any doubt the most accessible source of information concerning the composition and metabolic activity of the colon microbiota (Macfarlane and Macfarlane, 2004). Nevertheless, fecal microbial diversity is not considered representative for the entire colon ecosystem; at its best, it can provide relevant information regarding the composition of lumenal bacterial communities growing in the distal colon (Zoetendal et al., 2002; Zoetendal et al., 2006). Moreover, most feces-based studies focusing on the diversity of the colon microbiota still tend to start from the premise of colon bacteria occurring as independent individual cells, growing in suspension in a nutrient-rich semi-fluid environment. However, there is microscopic evidence that bacteria in the human colon commonly occur in microcolonies or disparate associations with other species on the surfaces of particulate materials such as food residues (Macfarlane and Dillon, 2007). While interindividual differences might be the main factor contributing to the diversity of the whole human colon microbiota, regional intraindividual variations make a convincing runner-up (Eckburg et al., 2005).

Formation of independent functional microbial entities or biofilms is a common phenomenon in microbial ecosystems. It allows microorganisms to develop coordinated multicellular behavior, both on an intra- and interspecies level, often through quorum sensing (bacterial cell-cell communication through the production and detection of diffusible signaling molecules, resulting in collectively controlled gene expression and synchronized group behavior) (Lazdunski et al., 2004; Salmond et al., 1995). Biofilm communities frequently possess phenotypic features unknown to the individual members, such as a highly efficient substrate metabolism, increased resistance to antibiotics, and decreased pH susceptibility. Moreover, microbial colon biofilms are thought to exhibit a greater resistance to the host's defensive immune system than their non-adherent counterparts (Macfarlane and Dillon, 2007). Although close spatial relationships between microorganisms on surfaces often limit growth by slowing down mass transfer and thus enhancing the effects of nutrient depletion, this drawback becomes negligible by the advantages offered by interbacterial metabolic communication (Macfarlane et al., 2000).

To this date, little is known concerning the formation, structure, and metabolic properties of biofilms in the human large intestine (Kleessen and Blaut, 2005; Macfarlane and Dillon, 2007). The mechanisms involved in attachment, co-aggregation, and quorum sensing that might be relevant to large-intestinal biofilm formation remain largely unexplored (Macfarlane and Dillon, 2007). However, as

colon diversity studies indicate the coexistence of distinct sub-ecosystems in the human large intestine (Eckburg et al., 2005), biofilm formation might be of key importance for large-intestinal ecosystem stability and host health.

16.2.5.1 The Lumenal Sub-Ecosystem

The decrease in nutrient availability combined with the increase in pH that characterize the colon lumen environment upon food passage through the large intestine, are thought to have a determining influence on lumenal species composition (Macfarlane and Cummings, 1991). While bifidobacteria and species belonging to clostridial clusters IV and XIVa are presumably more abundant in the proximal colon, the numerical importance of bacteroides and proteolytic *Firmicutes* is thought to increase toward the distal part (Dethlefsen et al., 2006; Walker et al., 2005). However, incomplete peristalsis permitting backflow and mixing contributes to the complexity of this image (Ley et al., 2006), as does the development of lumenal biofilms (Macfarlane and Dillon, 2007).

Lumenal biofilms are composed of bacteria adhering to particulate food residues passing through the gut. They are thought to play a key role in the rapid initial colonization of particulate substances entering the human colon, hence contributing to the stability of the gut ecosystem. The particularity of such biofilms – as well as of mucosal biofilms discussed below – lies in the transient character of the substrates they attach to, which makes them clearly distinct from communities growing on inert sites such as tooth surfaces or catheters (Flint, 2006). Notwithstanding the fact that current understandings of the primary development of such lumenal biofilms are still limited, two recent studies provided valuable information concerning their nature and composition, although presenting different – almost contrasting – results (Macfarlane and Macfarlane, 2006; McWilliam Leitch et al., 2007).

A first study focused on bacteria adhering to particulate matter found in human feces (Macfarlane and Macfarlane, 2006). It revealed that approximately 5% of the bacterial cell mass in the lumen of the large intestine is strongly adherent to the surface of partly digested food particles, with a considerably higher percentage loosely attached. Surprisingly, the composition of these adhering communities was found to be phenotypically similar to that of the unattached populations, with bacteroides and bifidobacteria predominating. In this study, the nature and composition of the insoluble substrates have not been determined. Unfortunately, bacterial identification has been carried out using a culture-dependent approach,

which could explain uncommon composition of the fecal microbiota reported. Fermentation experiments avoiding this culturability bias and using a variety of complex carbohydrates as substrates for both biofilms and non-adhering communities revealed that biofilm populations excel in polysaccharide breakdown, while their free-living counterparts prefer oligosaccharides as an energy source. Acetate is the main fermentation product produced by the biofilm communities, while a higher butyrate production has been noted for the non-adherent bacterial consortium.

A second study concentrated on the selective colonization of insoluble substrates by human fecal bacteria (McWilliam Leitch et al., 2007). Fecal samples from four individuals have been used in different fermentation experiments with wheat bran, high amylose starch, and porcine gastric mucin as added substrates. As culture-independent identification techniques have been applied, the detected proportion of butyrate-producing colon bacteria belonging to clostridial clusters IV and XIVa is far greater than in the study mentioned above, both in the fecal material used as inoculum and in the microbial communities recovered from the insoluble substrates. The study revealed the determining influence of the host as well as the nature of the substrate on biofilm formation. Overall, it could be concluded that bran surface is mainly covered by clostridial cluster XIVa bacteria and *Bacteroides* spp. On mucin, the bacterial species most commonly recovered are related to *Bifidobacterium bifidum* and *Ruminococcus lactaris*. On starch, *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, and *Eubacterium rectale* are most abundant. The fact that *Bacteroides* spp. have not been found to dominate the starch-attached bacterial populations is rather surprising, given the well-described arsenal of starch-degrading mechanisms that can be deployed by species such as *Ba. thetaiotaomicron* (Bjursell et al., 2006; Xu et al., 2003). The authors speculated that such species depend to a large extent on the availability of solubilized polysaccharides that are released by the activities of primary colonizing bacteria. In contrast with the results of an earlier study focusing on fermentation of soluble carbohydrates by human colon-derived microbial communities (Walker et al., 2005), pH did not influence the composition of the adherent microbiota during fermentation of insoluble substrates, a clear manifestation of a biofilm effect. Specific differences attributed to the composition of the donor microbiota could be noted throughout all fermentation experiments. Colonization of starch was, for example, dominated by *Bifidobacterium* spp. for two donors, while for the remaining two a consortium of *E. rectale* and *R. bromii* had the upper hand. This observation suggests that the interactions between the latter two species might be cooperative, whereas those

between them and *Bifidobacterium* spp. are presumably competitive (McWilliam Leitch et al., 2007).

16.2.5.2 Mucosal Biofilms

The human gastrointestinal tract is lined with a perpetually and rapidly renewing epithelium with the surface of a tennis court, shedding around 2×10^6 to 5×10^6 cells min^{-1} into the colon lumen (Croft and Levitan, 1970). The mucus gel layer overlying this gastrointestinal epithelium is the anatomical site at which the first host-colon bacteria encounters take place (Deplancke and Gaskins, 2001). Considered an integral component of the colon ecosystem, the mucus layer acts as a medium for protection, lubrication, and transport between the luminal contents and the epithelial lining. It is composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells. The thickness of the mucus layer increases gradually from the proximal colon up to the rectum (Deplancke and Gaskins, 2001). Mucin as well as epithelial cells carry carbohydrate chains that can act as receptors for binding of microorganisms and as potential energy sources for them (Flint, 2006). Due to the abundance of mucins as an energy source in the colon ecosystem, the human large intestine harbors some specialized mucin degraders, including *Akkermansia muciniphila*, *Ba. bifidum*, and *Ruminococcus torques* (Derrien et al., 2004; Flint, 2006; Hoskins, 1993). As the habitat of these species remains currently unclear, it is not certain whether they are involved in mucosal biofilm development. Other species definitely appear to grow in a close relationship with the colon mucosa; for example, *Ba. thetaiotaomicron* has been shown to induce epithelial cells to augment the production of fucose, a primary component of mucin, hence meeting its own nutritional needs (Hooper and Gordon, 2001; Hooper et al., 1999).

Studies concerning the mucosa-associated colon community and, more specifically, its distribution along the human large intestine are rather scarce, mostly due to practical and ethical limitations (Macfarlane and Dillon, 2007). Moreover, biopsies and surgical material are usually obtained from diseased individuals or from patients who have received antibiotic therapy, and from those whose colons have been washed-out before endoscopy or colonoscopy (Macfarlane and Dillon, 2007; Zoetendal et al., 2006). As a consequence, microbial biofilms found attached to these tissues may not be representative for the composition of communities found in healthy subjects.

Bacterial communities growing on the colon epithelium as well as those colonizing the mucus layer are thought to be more subject to a variety of host factors than their luminal counterparts. Besides general limitations such as nutrient availability, imposed on the whole of the large-intestinal ecosystem, mucosal colon biofilms are highly dependent on the rate of synthesis and the chemical composition of mucus, epithelial turnover rates, and disposability of adhesion sites. Moreover, bacteria growing in a close association with the human body are directly exposed to a stringent control by components of both the innate and the adaptive immune system (Macfarlane and Dillon, 2007).

Recent studies on the diversity of mucosa-associated biofilms in the human large intestine showed that the host specificity of the colon microbiota is reflected in the composition of mucosal biofilms (Eckburg et al., 2005; Frank et al., 2007). Molecular analysis of mucosal populations within an individual host demonstrated that most bacteria found on the colon epithelium belong to the same phylogenetic groups as those encountered in fecal material. *Bacteroidetes* and *Firmicutes* belonging to clostridial clusters IV and XIVa appear to be most abundant. Notwithstanding this superficial phylogenetic overlap, statistical diversity analyses reveal significant differences between the mucosal and luminal colon microbiota. Although some patchiness and heterogeneity has been reported (Eckburg et al., 2005), colon mucosa-associated bacterial populations are uniformly distributed along the gastrointestinal tract (Zoetendal et al., 2006).

Microscopic investigation of the three-dimensional structure of mucosal biofilms has shown that bacteria are present throughout the entire colon mucus layer, although they are usually not found in healthy crypts (Macfarlane et al., 2004). Live/dead staining of the bacterial structures (microcolonies) observed indicate that bacteria are actively growing in the mucus layer and that their presence is not only a result of passive transfer of cells from fecal material in the colon lumen (Macfarlane et al., 2004).

From the proximal to the distal colon, the human large-intestinal ecosystem is characterized by a gradual depletion of fermentable carbohydrates (Macfarlane et al., 1992). This depletion is translated in a rise in pH, a slower microbial turnover, and a lower metabolic activity of the microbiota present upon colon transit (Macfarlane and Cummings, 1991). However, this gradient does not seem to affect every colon sub-ecosystem to the same degree. Mucosal biofilms appear to maintain relative similar compositions and structures throughout the entire large intestine (Lepage et al., 2005). The limited bacterial species that can survive in close juxtaposition with the large-intestinal epithelium (due to the inherent

selectivity of the habitat) are most probably spared from abrupt changes in pH by the buffering influence of the colon epithelium and the constant uptake of fermentation products by epithelial cells (Macfarlane and Cummings, 1991). Moreover, they are constantly provided with mucin as a fermentable energy source (Macfarlane et al., 2004). In a dynamic environment, the colon epithelial surface can be regarded as a beacon of stability, ensuring survival of a strictly selected resident microbiota.

16.3 Dietary Carbohydrate Metabolism in the Human Colon

Growth and metabolic activity of the colon microbiota are highly dependent on nutrient availability. In the human large intestine, carbon and energy requirements of the resident bacterial populations are met by a continuous supply of fermentable substrates from dietary, microbial, and human origin (▶ [Figure 16.1](#)) (Macfarlane and Cummings, 1991). The host contribution to the intestinal energy metabolism consists mainly of body secretions such as pancreatic enzymes and mucins, but also sloughed epithelial cells become available as substrates for colon fermentations. The microbiota contributes to the colon energy cycle by acting as its own energy storage (Bäckhed et al., 2005): around 50% of the bacteria encountered in human feces are either damaged or dead, indicating that a substantial part of the intestinal microbial mass is in fact available as an energy source for bacterial recycling (Ben-Amor et al., 2005). Moreover, some members of the human colon microbiota have been shown to produce sugar polymers, which can act as a fermentable substrate for other intestinal bacteria, hence contributing to the complexity of the colon nutrient cycle (Salazar et al., 2008). However, the most abundant carbon and energy source present in the human colon consists of complex carbohydrates and, to a lesser extent, fats and proteins of food origin that have (partially) escaped digestion in the upper part of the gastrointestinal tract (Macfarlane and Cummings, 1991).

16.3.1 Substrate Availability

It has been estimated that – for a person consuming a Western diet – the amount of undigested carbohydrates that reach the colon varies between 20 and 65 g day⁻¹ (Macfarlane and Cummings, 1991). Most of these carbohydrates are

from plant origin and belong to a heterogeneous group of food ingredients known as dietary fibers: poly- and oligosaccharides of plant origin that resist both hydrolysis by mammal digestive enzymes and absorption in the small intestine, but that are at least partially hydrolyzed and fermented by the colon microbiota (Roberfroid, 2005a). The dietary fiber fraction of the human diet mainly comprises plant cell-wall polysaccharides, but it also covers storage carbohydrates and substances released by plants in response to injuries. From a chemical point of view, it includes cellulose, hemicelluloses, pectins, gums and mucilages, mixed-linkage β -glucans, resistant starch, and inulin (Roberfroid, 2005a). The largest dietary fiber fraction that enters the colon is made up of resistant starch. Daily, between 8 and 40 g of physical inaccessible, granulated, or retrograded starch reaches the large intestine undigested and becomes available as a substrate for colon fermentation (Macfarlane and Cummings, 1991). Concentrations of free monosaccharides in the colon are generally presumed to be low (Flint, 2006), although it has been shown that they may play an important role as short-living substrates involved in cross-feeding interactions (Falony et al., 2006).

16.3.2 Functional Redundancy

As plant poly- and oligosaccharides make up the largest fraction of the diet-derived energy sources in the human colon, the possession of an enzymatic arsenal necessary to tackle these substrates is an important factor for the survival of large-intestinal microorganisms. Not surprisingly, glycoside hydrolases, esterases, and other enzymes required to degrade plant polysaccharides are found in a wide range of colon bacteria, including *Bacteroides* spp., *Bifidobacterium* spp., and *Firmicutes* belonging to clostridial clusters IV and XIVa (Ramsay et al., 2006; Schell et al., 2002; Xu et al., 2003). This functional redundancy persists even at the strain level: in the few genomes of colon bacteria studied up to now, multiple gene copies of proteins involved in anchoring, uptake, and degradation of carbohydrates have been detected (Bäckhed et al., 2005; Klijn et al., 2005).

Complex carbohydrates entering the human colon vary among each other in chemical composition, degree of polymerization (DP), structure, accessibility (association with other molecules), and solubility (Flint et al., 2008). Although this substrate diversity is thought to create a vast array of microbial niches, a driving force for colon microcommunity differentiation, many common gut-colonizing bacteria appear to be generalists – bacterial strains capable to degrade

a variety of substrates – with comparable growth abilities on overlapping ranges of substrates (Dethlefsen et al., 2006). The key to successful proliferation in the colon seems to lie in combining a broad range of enzymatic carbohydrate breakdown activities with adequate oligosaccharide uptake systems and, optionally, an effective anchoring mechanism (Flint et al., 2008). Niche adaptation among carbohydrate-degrading species is mainly reflected by the organization and regulation of the corresponding gene products (Flint, 2004). The presence of generalists provides the colon ecosystem with a two-leveled strategy to respond to both quantitative and qualitative changes in diet composition (Louis et al., 2007). At the strain level, bacterial metabolic regulation allows generalist microorganisms to meet alterations in substrate nature or supply rate by switching to alternative metabolic routes, often reflected by fluctuations in the bacterial fermentation product profile (Macfarlane and Macfarlane, 2003; Van der Meulen et al., 2004, 2006b). On the community level, due to the widespread functional redundancy among related, habitat-specialized species, even sustained changes in dietary input will provoke only shallow changes in bacterial composition (Bäckhed et al., 2005). Both interrelated mechanisms contribute to the stability of the colon ecosystem.

Ba. thetaiotaomicron is often presented as a perfect example of a successful, flexible, niche-adapted, human commensal with a wide carbohydrate consumption range (Bjursell et al., 2006; Comstock and Coyne, 2003; Xu et al., 2003). This species, which has only been isolated from human and rodent intestines and feces up to now, has been reported to make up 6% of the colon microbiota (Eckburg et al., 2005). A large part of its 6.3-Mb genome – sometimes referred to as its ‘glycobiome’ – encodes for 163 paralogs of outer-membrane, starch-binding and –importing proteins, 226 predicted glycoside hydrolases (amylases, fructofuranosidases, pullulanase, etc) and 15 polysaccharide lyases (e.g., pectate lyase) (Xu et al., 2003; Xu and Gordon, 2003). Moreover, the species has been shown to be able to redirect its carbohydrate-harvesting activities according to nutrient availability (Bjursell et al., 2006; Sonnenburg et al., 2005). This provides *Ba. thetaiotaomicron* not only with the weapons necessary to grow on a wide range of undigested polysaccharides, but also to adapt to changes in the composition of the human diet, allowing it to survive in and dominate the densely populated human intestinal ecosystem (Louis et al., 2007). A comparable, though less extensive, genetic potential for colon substrate metabolism has been shown to be present in *Bifidobacterium longum* NCC2705. This strain is equipped with more than 40 glycosyl hydrolases (amylases, fructofuranosidases, etc) that are predicted to be involved in the degradation of higher order oligosaccharides

(Klijn et al., 2005; Schell et al., 2002). Also, it possesses at least nine transport systems involved in the uptake of oligofructose (Parche et al., 2007).

16.3.3 Niche Specialization

Notwithstanding the widespread functional redundancy among colon bacteria regarding carbohydrate degradation, some niches require a higher degree of specialization. Efficient degradation of recalcitrant insoluble polysaccharides such as plant cell-wall components and starch granules, for example, is dependent on substrate colonization and formation of degradative biofilms (Macfarlane and Macfarlane, 2006). Surface adhesion, the first step of substrate colonization, is an up to now poorly understood process. It is thought to be a highly specific chain of events involving diverse substrate-binding modules located in enzymes and structural proteins, as well as the carbohydrate moieties of glycoproteins (Flint et al., 2008).

Presently, degradation of insoluble plant materials by human colon bacteria has not been characterized in detail (Flint et al., 2008). However, similar interactions as those thought to be active in the human large intestine have been described for species inhabiting the rumen ecosystem. Cellulose degradation by *Ruminococcus flavefaciens*, for example, involves the production of an elaborate cell surface-anchored cellulosome, that is thought to play a key role in the breakdown of plant cell walls (Rincon et al., 2005). Such cellulosomes are discrete, extracellular, multi-component, and multi-enzyme complexes that provide enhanced synergistic activity among the different resident enzymes to efficiently deconstruct cellulosic and hemicellulosic components of plant cell walls (Flint et al., 2008). Related cellulolytic bacteria are reported to be present in the human colon of methane-producing individuals (Robert and Bernalier-Donadille, 2003).

16.3.4 Secondary Degradation

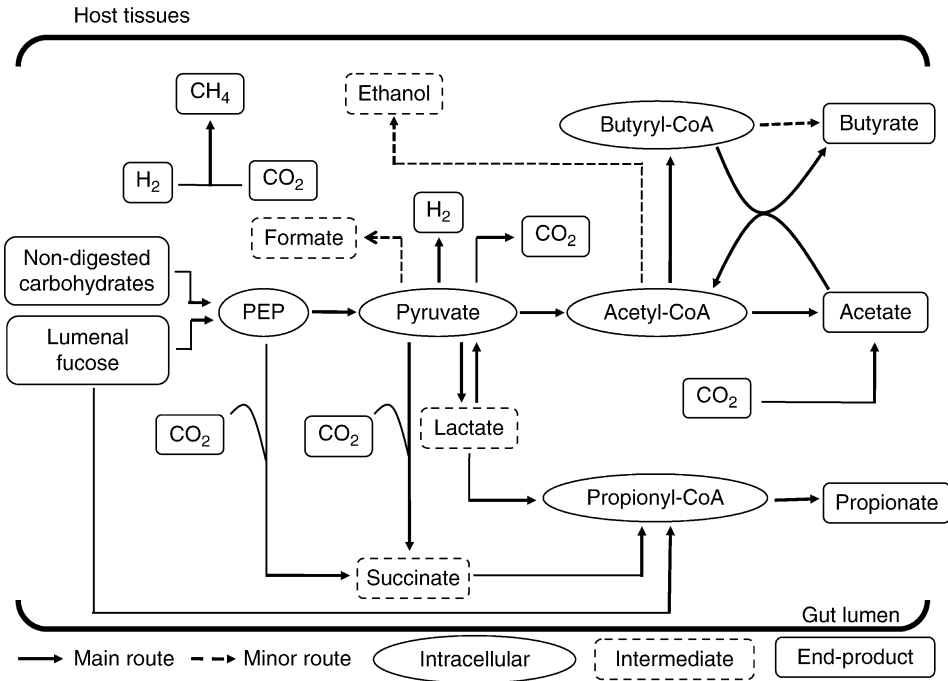
Degradation of insoluble plant components or large polysaccharides generally provokes physical changes to the available substrates, for example regarding their solubility or physical accessibility. Moreover, such breakdown processes are often accompanied by the release of oligosaccharides in the microenvironment surrounding the degradation site, creating possibilities for opportunistic competitors (Flint et al., 2007). The availability of more readily fermentable substrates,

such as monosaccharides and short oligosaccharides, enhances biofilm maturation. Mature microbial consortia covering transient degradable substrates, such as food residues, are composed of primary degraders, growing directly on the surface of the substrates, surrounded by co-aggregating or even non-adhering secondary colonizers, feeding on partially hydrolyzed breakdown products (Flint et al., 2008; Macfarlane and Macfarlane, 2006). This mechanism of cross-feeding, i.e., the provision of breakdown products of polysaccharides as secondary substrates after partial hydrolysis by primary degraders, has been demonstrated for rumen bacteria to explain fiber digestion in ruminants (Dehority, 1991; Flint et al., 2007) and has recently been described for colon isolates growing *in vitro* on indigestible oligosaccharides (Belenguer et al., 2006; Falony et al., 2006).

To avoid opportunistic substrate competition by secondary degraders, several colon bacteria developed strategies to ensure that they remain the main beneficiaries of their hydrolytic activity. Starch degradation by *Ba. thetaiotaomicron*, for example, initiates with outer membrane binding of soluble starch molecules (Cho and Salyers, 2001; Xu et al., 2003). A second step consists of limited polysaccharide degradation using a periplasmic membrane-bound glycoside hydrolase (neopullulanase), followed by the uptake of starch debris ranging in size from maltose to maltoheptaose. Most of the glycosidic activity displayed by *Bacteroides thetaiotaomicron* is located in the periplasm, where oligosaccharides are further degraded before being transported into the cell (Salyers, 1984). The ability to take up large oligosaccharides with a DP up to eight has not only been reported for *Ba. thetaiotaomicron*, but is also thought to be present in some bifidobacteria (Schell et al., 2002). Uptake and subsequent degradation of substrates offers bacteria a substantial advantage, as intracellular breakdown is usually not accompanied by the release of large amounts of short oligosaccharides or monosaccharides in the cellular environment (Van der Meulen et al., 2006b).

16.3.5 Metabolic Cross-Feeding

The major end-products of bacterial fermentation in the large intestine are SCFA and gases (🔗 [Figure 16.2](#)) (Macfarlane and Cummings, 1991). The principal SCFA that result from carbohydrate fermentation are acetate, propionate, and butyrate, although formate, valerate, and caproate are also produced, albeit in lesser amounts (Macfarlane and Macfarlane, 2003). Other fermentation products, including ethanol, lactate, and succinate, do not accumulate in the colon



■ **Figure 16.2**

Principal substrate fluxes during colon carbohydrate fermentation.

ecosystem of healthy individuals. They serve as intermediates in metabolic cross-feeding interactions between colon bacteria and are metabolized to SCFA to varying extents. Combined total concentrations of acetate, propionate, and butyrate range between 90 and 130 mmol (kg gut contents)⁻¹ (Cummings et al., 1987); a total daily production of 400 mmol SCFA has been suggested (Cummings et al., 1989). Although SCFA production rates vary throughout the gut and concentrations are highest in the proximal colon, the acetate:propionate:butyrate ratio is similar in different regions of the large intestine, namely about 57:22:21 (Cummings et al., 1987). However, SCFA levels in bowel contents are not absolute; they merely reflect the balance between bacterial production and colon absorption (Macfarlane and Macfarlane, 2003).

Gas production is an integral part of colon fermentation processes. Gases generated are H₂, CO₂, and, in up to 50% of healthy individuals living on a Western diet, CH₄ (Macfarlane and Cummings, 1991). Gas generated in the colon is either excreted in breath or expelled as flatus, although CO₂ and H₂ also serve as intermediates in cross-feeding interactions between colon bacteria. The total

amount of gas produced on a daily basis is diet-dependent, with estimated values ranging between 0.5 and 4 l day⁻¹. Flatus gas has an average composition of 68% N₂, 16% H₂, 9% CO₂, 6% CH₄, and 2% O₂ (Macfarlane and Cummings, 1991).

16.3.5.1 Production and Consumption of SCFA

Acetate. With concentrations of approximately 70 mmol (kg contents)⁻¹ in the human cecum, lowering to 50 mmol (kg contents)⁻¹ in the sigmoid colon, acetate is quantitatively the most important end-product of colon fermentation processes (Cummings et al., 1987). The microbial origins of colon acetate are legion: not only is it produced by nearly all heterotrophic gut anaerobes (▶ Table 16.1), up to one-third of the colon acetate pool originates from reductive acetogenesis by, for example, *Ruminococcus hydrogenotrophicus* (Miller and Wolin, 1996). As an intermediate in cross-feeding interactions between colon bacteria, acetate plays a key role in large-intestinal butyrate production. Acetate consumption is shown to be common among butyrate-producing gut bacteria, whereas it remains rare in other functional groups of colon inhabitants (▶ Table 16.1) (Barcenilla et al., 2000). It has been demonstrated *in vitro* that acetate can contribute up to 90% of the carbon needed for butyrate production by the human fecal microbiota, depending on the primary substrate administered (Duncan et al., 2004a).

Propionate. Propionate concentrations in the human large intestine vary between 25 mmol (kg contents)⁻¹ in the cecum and 20 mmol (kg contents)⁻¹ in the sigmoid colon (Cummings et al., 1987). For many years, colon propionate production has been attributed to *Bacteroides* spp. (Macfarlane and Cummings, 1991). However, the metabolic outcome of bacteroidal substrate degradation is highly dependent on nutrient availability and CO₂ concentrations (Macy et al., 1978; Salyers, 1984). When sufficient carbohydrates are present, there is a reduced need for *Bacteroides* spp. to decarboxylate succinate to propionate, and the former metabolite tends to accumulate (Salyers, 1984; Van der Meulen et al., 2006b). It remains unclear which is the main end-product of bacteroidal metabolism in the highly competitive colon environment (Macfarlane and Macfarlane, 2003). Recently, it has been demonstrated that clostridium cluster IX bacteria, responsible for propionate production in other gastrointestinal ecosystems, such as the rumen, can make up to 13% of the total fecal microbiota (Walker et al., 2005). The composition of this bacterial subgroup in the human colon remains at present poorly characterized. However, as it is thought to include *Megasphaera* spp.

and *Veillonella* spp., it could be involucrated in conversion of both succinate and lactate to propionate (Bourriaud et al., 2005; Flint, 2006). Propionate has been reported to have an inhibitory effect on hepatic fatty acid synthesis, making stimulation of large-intestinal propionate production a possible strategy in the struggle against obesity (Delzenne et al., 2002).

Butyrate. With cecal and sigmoid colon concentrations of 26 and 18 mmol (kg contents)⁻¹ respectively, butyrate is quantitatively the third major end-product of large-intestinal fermentation processes (Cummings et al., 1987). Colon butyrate producers mainly belong to the *Firmicutes* division and can be situated in the clostridial clusters I, III, IV, XI, XIVa, XV, and XVI (Pryde et al., 2002). However, more than 90% of colon butyrate-producing microorganisms are in fact cluster IV and XIVa bacteria, related to *Faecalibacterium prausnitzii* and *E. rectale* or *Roseburia* spp., respectively (Barcenilla et al., 2000; Louis et al., 2007). Together, these two particularly abundant groups have been reported to constitute between 7 and 24% of the total colon microbiota in healthy individuals (Aminov et al., 2006; Hold et al., 2003; Louis et al., 2007). The pathway for microbial butyrate production involves the condensation of two molecules of acetyl coenzyme A (acetyl-CoA) and their subsequent reduction to butyryl-CoA (Diez-Gonzalez et al., 1999; Duncan et al., 2002a). For the final step of the pathway, the actual butyrate formation, two alternative metabolic routes have been described (► [Figure 16.2](#)). Butyrate can be produced using a butyrate kinase, as has been demonstrated in some strains of *Butyrivibrio fibrisolvens* (Diez-Gonzalez et al., 1999; Duncan et al., 2002a). Alternatively, a butyryl-CoA:acetyl-CoA transferase can move the CoA-moiety to external acetate, leading to the production of butyrate and acetyl-CoA, as is the case for *Roseburia intestinalis* (Duncan et al., 2002a; Louis et al., 2004). In the acetate-rich colon ecosystem, butyryl-CoA:acetyl-CoA activity has been proven common among butyrate-producing strains, in contrast to butyrate kinase activity (Louis et al., 2004). This makes (external) acetate a key intermediate for colon butyrate production.

16.3.5.2 Succinate, Lactate, and Ethanol

Succinate, lactate, and ethanol only figure in the human large intestine as intermediates of cross-feeding interactions between bacteria (Macfarlane and Macfarlane, 2003). Like for the end-products of colon fermentation, but perhaps more confronting and limiting, the study of their occurrence and metabolic fate in the large-intestinal ecosystem suffers greatly under the current lack of adequate *in vivo/in situ*

methods to determine their production rates (Morrison et al., 2006). Ethanol, for example, is regularly mentioned as a minor (end-)product of colon fermentation processes, but the concentration at which it occurs or its further degradation has never been investigated (Macfarlane and Macfarlane, 2003).

Lactate concentrations in the human large intestine decrease from 5 mmol (kg contents)⁻¹ in the cecum to 1.5 mmol (kg contents)⁻¹ in the sigmoid colon (Macfarlane and Cummings, 1991). Fecal lactate concentrations above 5 mM have been linked with disease conditions such as severe colitis (Vernia et al., 1988). Low lactate concentrations could be related with low large-intestinal production rates, but this seems to be contradicted by metagenomic analyses of the colon microbiome (Gill et al., 2006; Turroni et al., 2008). More likely, lactate is efficiently consumed by both propionate- and butyrate-producing colon inhabitants belonging to clostridial clusters IX and XIVa, respectively (Belenguer et al., 2006; Bourriaud et al., 2005; Duncan et al., 2004b), with butyrate production dominating at lower pH (Belenguer et al., 2007). Also, sulfate-reducing colon bacteria have been implicated in lactate removal (Flint et al., 2007).

Succinate concentrations remain constant throughout the human large intestine, with an average concentration of approximately 1 mmol (kg contents)⁻¹ (Macfarlane and Cummings, 1991). Considering the genetical potential present in the colon microbiome, the latter reflects efficient turnover rather than low production rates (Gill et al., 2006; Turroni et al., 2008). The metabolic fate of colon succinate is presently unknown (Walker et al., 2005). However, it should be noted that *Bacteroides* spp., possible sources of colon succinate, become more dominant at higher pH (Walker et al., 2005). The latter implies a more abundant presence of *Bacteroides* spp. in the distal colon, where the fermentable carbohydrate availability is limited (Macfarlane et al., 1992). Under these circumstances, *Bacteroides* spp. tend to produce more propionate than succinate (Macy et al., 1978; Salyers, 1984). Succinate production by bifidobacteria – a genus that is thought to be generally represented in the proximal colon – has been demonstrated, but only in minor amounts (Van der Meulen et al., 2006a).

16.3.5.3 Hydrogen Gas Metabolism

Hydrogen gas accumulation is a determining factor in the balance and outcome of anaerobic fermentation processes (Stams, 1994). High partial H₂ pressure has been reported to affect growth rates and polysaccharide fermentation and SCFA production by H₂-producing microorganisms (Rychlik and May, 2000).

As many colon bacteria – including butyrate-producing members of the *Firmicutes* division – produce H_2 during carbohydrate degradation, the latter has important consequences for metabolic processes within the colon ecosystem (Duncan et al., 2002b, 2006; Schwartz et al., 2002).

The two main enzymatic complexes linked with H_2 production by intestinal bacteria involve a pyruvate:ferredoxin-oxidoreductase and a NADH:ferredoxin-oxidoreductase, both coupled with a hydrogenase (Macfarlane and Macfarlane, 2003). While the latter enzymatic route is thought to be the most common among colon bacteria, it is – in contrast with the former – endergonic and requires low partial H_2 pressure to be thermodynamically feasible. Under conditions of high partial H_2 pressure, intestinal bacteria are forced to equilibrate their redox balances through the production of more reduced metabolites, including succinate, lactate, butyrate, and ethanol (Bourriaud et al., 2005). The formation of more oxidized end-products, such as acetate, is considered energetically more interesting, as it is usually associated with ATP production (Macfarlane and Macfarlane, 2003). In the colon ecosystem, H_2 is either released as gas or consumed by H_2 -utilizing species, in casu methanogenic Archaea, sulfate reducers, and acetogens (Macfarlane and Cummings, 1991).

Notwithstanding the fact that acetogens show a lower affinity for hydrogen gas than methanogens or sulfate reducers, acetogenesis has been reported to provide a substantial part of the colon acetate reserve (Miller and Wolin, 1996). Acetogenesis is thought to be the dominant H_2 -consuming process in the proximal part of the human colon, where acetogens might be favored by the slightly acidic conditions (Bernalier et al., 1996a; Macfarlane and Cummings, 1991). Recently, efficient cross-feeding has been demonstrated *in vitro* between *R. intestinalis* (a clostridial cluster XIVa butyrate producer) and *R. hydrogenotrophicus* (an acetogen) when growing on xylan (Bernalier et al., 1996b; Chassard and Bernalier-Donadille, 2006). The substrate is initially degraded by *R. intestinalis*, producing CO_2 and H_2 , which in turn serve as substrates for *R. hydrogenotrophicus*. The latter produces acetate, an indispensable co-substrate for butyrate production by *R. intestinalis*, resulting in xylan degradation without net H_2 production (Chassard and Bernalier-Donadille, 2006).

Concerning methanogens and sulfate reducers, it is not clear whether both groups of bacteria can coexist or are mutually exclusive (Doré et al., 1995; Flint et al., 2007). Competition for hydrogen gas between sulfate-reducing and methanogenic bacteria may explain why some individuals produce methane while others do not. In fecal slurry, sulfate reducers outcompete methanogens, but the outcome of this competition is thought to be highly sulfate-dependent

(Gibson et al., 1988). However, little is known about the availability of sulfate in the human colon (Macfarlane and Cummings, 1991). As the dominating route for hydrogen gas disposal in a given individual influences the competitive balance between other colon bacteria, the result of competition for H₂ might have serious implications for the large-intestinal ecosystem (Robert and Bernalier-Donadille, 2003). In addition, sulfate reduction has potential deleterious consequences on gut health via the formation of toxic sulfide, which has been implicated with the development of ulcerative colitis (Flint et al., 2007; Pitcher et al., 2000).

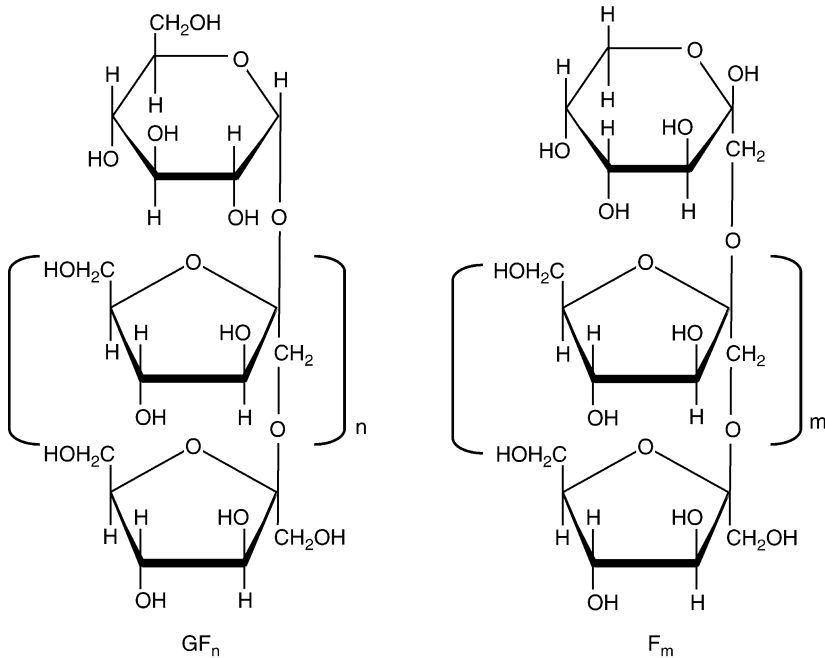
16.4 Ecological Background of Colon Inulin-Type Fructan Fermentation

More than a decade of intensive research has radically altered both scientists' and consumers' perception of the role and importance of the human colon microbiota and the large-intestinal ecosystem. The fundamentals of this new perception can be summarized as follows: (1) host health and well-being are influenced by the colon microbiota (Macfarlane and Cummings, 1991; Roberfroid, 2005b), (2) the nature of a healthy or balanced colon microbiota is definable (Louis et al., 2007; Macfarlane et al., 2006), and (3) the composition and/or metabolic activity of the colon microbiota can be influenced (transiently) through changes in the diet (Rastall et al., 2005). Notwithstanding the fact that the large-intestinal ecosystem remains largely unexplored (Duncan et al., 2007b; Eckburg et al., 2005), different strategies to influence host health by managing the composition and/or activity of the colon microbiota through the diet have emerged from these understandings (Duncan et al., 2007a; FAO/WHO, 2001; Gibson and Roberfroid, 1995; Makras et al., 2004; Rastall et al., 2005). Although hard to define, the goal of such dietary interventions is to establish an optimally balanced colon microbiota, which is generally believed to be predominantly saccharolytic, comprising significant numbers of bifidobacteria and lactobacilli (Macfarlane et al., 2006).

A well-established strategy to alter the colon ecosystem consists in the use of prebiotics, selectively fermented non-digestible food ingredients that allow specific changes in the composition and/or activity of the gastrointestinal microbiota, which confer benefits upon host well-being and health (Gibson et al., 2004; Gibson and Roberfroid, 1995). At the origin of the development of the prebiotic concept lies the observation of the stimulative effect of inulin-type fructans on the fecal *Bifidobacterium* population during *in vitro* experiments (Gibson and Roberfroid, 1995; Gibson and Wang, 1994; Wang and Gibson, 1993), an observation that was

later largely confirmed by *in vivo* trials (Roberfroid, 2005c). Although the prebiotic properties of other non-digestible food ingredients have been acknowledged by now (Gibson et al., 2004), oligofructose and inulin still remain best studied and have gained themselves a status of model prebiotics (Bosscher et al., 2006; Roberfroid, 2005c).

Inulin-type fructans are linear *D*-fructose polymers linked by $\beta(2-1)$ -glycosidic bonds (F_m -type), often with a terminal glucose moiety that is linked by an $\alpha(1-2)$ -glycosidic bond (GF_n -type), as in sucrose (► Figure 16.3). They are present in significant amounts in several fruits and vegetables (e.g., onion, banana, garlic, leek, chicory) (Makras et al., 2004). On an industrial scale, inulin-type fructans are usually extracted from chicory roots (Roberfroid et al., 1998).



► Figure 16.3

Chemical structure of inulin-type fructans. F, fructose; G, glucose. In the present manuscript, the terms inulin and oligofructose – obtained through partial enzymatic hydrolysis of chicory inulin – are used to refer to two distinct fractions of native chicory inulin, both containing F_m - as well as GF_n -type polymers. The DP of the oligofructose fraction varies between 2 and 10 with an average DP of 4, whereas that of inulin can be 60 or even more, with an average DP exceeding 23.

Inulin-type fructans are neither digested nor absorbed in the human upper gastrointestinal tract (Molis et al., 1996). They reach the colon virtually intact, where they are selectively fermented by the large-intestinal microbiota and mainly converted not only to SCFA, such as acetate, propionate, and butyrate, but also to other organic acids (e.g., lactate) and gases (H_2 and CO_2) (Alles et al., 1996). Although the physiological effects of inulin-type fructan consumption are extensively documented, their impact on the colon ecosystem is less understood. The most pronounced effects of their availability, as substrates of microbial fermentation, on the colon ecosystem are an increase in bifidobacterial numbers, the so-called bifidogenic effect (Gibson et al., 1995; Gibson et al., 2004; Roberfroid et al., 1998), and an enhancement of large-intestinal butyrate production, the so-called butyrogenic effect (Campbell et al., 1997; Le Blay et al., 1999; Morrison et al., 2006). These bifidogenic and butyrogenic effects are considered beneficial for the host's health, but their combination is remarkable, as bifidobacteria are not able to produce butyrate (Makras et al., 2006; Van der Meulen et al., 2006a; Van der Meulen et al., 2004).

16.4.1 The Bifidogenic Effect

Although stimulation of bifidobacteria by inulin-type fructans has been studied extensively for more than 15 years through both *in vitro* and *in vivo* trials, some rather elementary questions still await satisfactory answering (Macfarlane et al., 2006). It is, for example, not clear how bifidobacteria [$<3\%$ of the large-intestinal microbiota (Frank et al., 2007)] manage to get the upper hand in the highly competitive human colon ecosystem when inulin or oligofructose are added to the diet, while it has been demonstrated that also *Lactobacillus* spp. (Goh et al., 2006; Kaplan and Hutkins, 2000, 2003; Makras et al., 2005), *Roseburia* spp. (Duncan et al., 2002b, 2006; Falony et al., 2006), *Bacteroides* spp. (Van der Meulen et al., 2006b), and some enterobacteria (Hartemink et al., 1997) can use at least oligofructose as a substrate for fermentation (Huebner et al., 2007). It has been suggested that (some) *Bifidobacterium* spp. are capable of intracellular or cell-associated degradation of oligofructose, providing them with a fermentation advantage (Falony et al., 2006, 2009a; Schell et al., 2002; Van der Meulen et al., 2006b). Non-bifidobacterial species, such as *Bacteroides* spp., are thought to degrade oligofructose extracellularly, making them less competitive when growing on this substrate (Falony et al., 2006; Van der Meulen et al., 2006b). Moreover, as

not all bifidobacteria are able to degrade inulin or even oligofructose to the same extent (Huebner et al., 2007; Rossi et al., 2005), it seems likely that not all bifidobacterial species benefit in the same way of the presence of inulin-type fructans as energy sources in the colon. However, most studies concerning the bifidogenic effect of inulin-type fructans tend, unfortunately, to consider the bifidobacterial colon population as a whole, not taking into account the interspecies differences that exist between various bifidobacteria (Macfarlane et al., 2006). Recently, the existence of four phenotypically distinct clusters among bifidobacteria, based on their inulin-type fructan breakdown capacities, has been revealed, probably reflecting niche-specific adaptation (Falony et al., 2009b). This rather limited variation has been hypothesized to influence the susceptibility of various bifidobacteria toward prebiotic stimulation by inulin-type fructans and their fitness to compete for these substrates in a complex environment such as the colon ecosystem.

Recent *in vitro* studies focussing on detailed kinetic analyses of oligofructose degradation by bifidobacteria as well as *Lactobacillus paracasei* subsp. *paracasei*, *Ba. thetaiotaomicron*, *Bacteroides fragilis*, and *R. intestinalis* have revealed remarkable differences between the bifidobacterial breakdown mechanism and that of species belonging to other genera (Falony et al., 2006; Makras et al., 2005; Van der Meulen et al., 2006b, 2004). Most bifidobacteria seem to degrade oligofructose in a preferential way, i.e., preferring the DP 2 fraction over the DP 3 and 4, only initiating the breakdown of a longer chain length fraction when shorter fractions are depleted. Furthermore, only a limited number of bifidobacteria appears capable of degrading the inulin fraction (Falony et al., 2009b; Rossi et al., 2005). Non-bifidobacterial species exhibit simultaneous degradation of all fractions, often combined with the release of large amounts of free fructose in the fermentation medium, as has been revealed *in vitro* for *L. paracasei* subsp. *paracasei* 8700:2 (Makras et al., 2005) and *Ba. thetaiotaomicron* LMG 11262 (Van der Meulen et al., 2006b). It has been suggested that accumulation of breakdown products of inulin-type fructans can be linked to extracellular degradation, which might prove to be less efficient than internal or cell wall-bound degradation in a highly competitive ecosystem such as the human colon (Falony et al., 2006; Van der Meulen et al., 2006b). The appearance of free fructose and shorter fractions of oligofructose in the growth environment, which become available to opportunistic competitors that are not able to break down inulin-type fructans themselves (Flint et al., 2007; Louis et al., 2007), seems to be inherent to extracellular degradation. Bifidobacteria capable of intracellular degradation would not suffer from this drawback, which would explain their selective

growth stimulation on inulin or oligofructose (Falony et al., 2006; Rossi et al., 2005; Van der Meulen et al., 2006b).

16.4.2 The Butyrogenic Effect

Many of the health-promoting effects attributed to oligofructose and inulin are at least partially due to their influence on the production of SCFA by the colon microbiota (Delzenne et al., 2002; Guarner, 2005; Nyman, 2002; Pool-Zobel, 2005; Weaver, 2005). In humans, colon fermentation of inulin-type fructans generally does not lead to a significant increase of fecal concentrations of SCFA or to a change in molar proportions of acetate, propionate, and butyrate (Nyman, 2002). The latter can be explained by the very efficient colon SCFA absorption (Macfarlane and Cummings, 1991; Macfarlane and Macfarlane, 2003). Only drastic dietary interventions appear to be reflected in colon SCFA concentrations (Walker et al., 2005). In contrast, *in vitro* and animal studies do show an enhancement of SCFA production by inulin and oligofructose (Djouzi and Andrieux, 1997; Wang and Gibson, 1993). In particular, fermentation of inulin-type fructans by the colon microbiota seems to cause an increase in butyrate formation (Campbell et al., 1997; Le Blay et al., 1999; Morrison et al., 2006). This butyrogenic effect was already known in the case of resistant starch fermentation (Brouns et al., 2002; Scheppach and Weiler, 2004). Butyrate is of key importance for colon health: it is not only the preferred energy source for the large-intestinal epithelium (Macfarlane and Cummings, 1991), but it also has important effects on the development of and the gene expression in intestinal cells (Mariadason et al., 2000; Scheppach and Weiler, 2004). In addition, it is thought to play a protective role against colorectal cancer and colitis (Hague et al., 1996; Hamer et al., 2008).

The link between consumption of inulin-type fructans, the bifidogenic effect, and the increase of butyrate production, the butyrogenic effect, in the colon has remained unclear for several years. Bifidobacteria produce lactate, acetate, formate, ethanol, and even minor amounts of succinate, but can not produce butyrate (Falony et al., 2006; Van der Meulen et al., 2006a). Furthermore, evidence of direct degradation of inulin-type fructans by butyrate-producing colon bacteria is scarce (Duncan et al., 2006; Duncan et al., 2003; Falony et al., 2006). Although it has become clear that the stimulatory effect of inulin-type fructans exceeds the large-intestinal *Bifidobacterium* population and affects other genera, including butyrate-producing bacteria belonging to the clostridial cluster

XIVa (Duncan et al., 2003; Langlands et al., 2004), it is generally believed that such species do not play a major part in colon fructan breakdown.

Cross-feeding between starch-degrading bifidobacteria and lactate-converting, butyrate-producing colon bacteria has been demonstrated (Duncan et al., 2004b). Similarly, recent studies regarding the combined bifidogenic/butyrogenic effect of inulin-type fructans focussed mainly on cross-feeding between bifidobacteria and butyrate-producing colon bacteria belonging to clostridial cluster XIVa. In the case of inulin-type fructans, two main types of cross-feeding have been demonstrated: one involving short oligosaccharides and monosaccharides released by *Bifidobacterium* spp. from the prebiotic substrate; the other one by means of consumption of end-products of bifidobacterial fructan fermentation such as acetate and lactate (Belenguer et al., 2006; Falony et al., 2006). *Eubacterium hallii* DSM 17630, not capable of fructan degradation, has been shown to efficiently convert lactate and acetate produced by *B. adolescentis* DSM 20083, when growing in coculture on oligofructose (Belenguer et al., 2006). *Anaerostipes caccae* DSM 14662^T survived in a coculture with *B. longum* BB536, when growing on oligofructose, by taking advantage of acetate produced and fructose released by the latter strain during substrate breakdown (Falony et al., 2006). *R. intestinalis* DSM 14610^T joined *B. longum* BB536 in oligofructose degradation, but only after the latter strain initiated breakdown and started to produce acetate, an indispensable co-substrate for growth of the former strain (Falony et al., 2006). The occurrence of such or related cross-feeding interactions is inevitable when inulin-type fructans or other prebiotics are added to the diet and make their way to the large intestine as substrates for colon fermentation. Prebiotic fermentation will inherently indirectly affect other groups of colon bacteria than the ones targeted, complicating any attempt to predict the full extension of their effects (Flint et al., 2007). Notwithstanding the often underestimated complexity of the impact of fermentation of prebiotics on the colon ecosystem, the bifidogenic effect and its associated beneficial consequences on human health and well-being, linked with for example inulin-type fructan consumption, remain beyond dispute (Bosscher et al., 2006).

Although the *in vitro* demonstration of cross-feeding between primary inulin-type fructan degraders and butyrate-producing colon bacteria is convincing, these model mechanisms can not be translated to the colon ecosystem without caution. Many factors contributing to the *in vivo* ecological background of colon inulin-type fructan breakdown that remain at present insufficiently explored will undoubtedly complicate this simplified *in vitro* vision on interspecies interactions (➤ [Table 16.2](#)).

■ **Table 16.2**

Ecological implications of colon inulin-type fructan degradation: unanswered questions (Bosscher et al., 2006; Roberfroid, 2005a; Rossi et al., 2005; Van der Meulen et al., 2006b)

<p>Which bifidobacteria are responsible for inulin-type fructan degradation?</p> <p>It is well documented that not all bifidobacteria are capable to degrade inulin-type fructans. Moreover, bifidobacterial inulin degradation is rare, indicating that bifidobacteria merely act as secondary degraders of oligofructose. Metabolite production is species-dependent: while some bifidobacteria produce lactate and acetate when growing on oligofructose, others produce acetate, formate, and ethanol solely. Furthermore, bifidobacterial metabolism is subject to drastic changes as a function of substrate degradability and fermentation rate. However, it remains presently unknown which bifidobacterial species play a key role in fructan degradation nor what their metabolic end-products are in the highly competitive colon environment.</p>
<p>What is the effect of increased H₂ production?</p> <p>Colon fermentation of inulin-type fructans results in an increase in gastrointestinal H₂ production. Changes of intestinal partial pressure of H₂ are thought to affect the metabolism of large groups of colon bacteria, including butyrate producers belonging to clostridial clusters IV and XIVa. Moreover, it may favor H₂-consuming microorganisms, including methanogens and sulphate reducers, in the colon environment. The extent and the implications of these changes in the colon microbiota are currently unknown.</p>
<p>What is the location of oligofructose and inulin degradation?</p> <p>Degradation of oligofructose is generally thought to take place in the proximal colon, while fermentation of the longer inulin chains would take place in the more distal parts of the colon. Composition of bacterial populations evolves alongside the large intestine, with less bifidobacteria and clostridial cluster IV and XIVa butyrate producers thought to be present toward the distal colon. However, it is not known what the effect of this translocation of fermentation site is on the nature and metabolic end-products of inulin-type fructan degradations.</p>
<p>What is the role of <i>Bacteroides</i> spp. in inulin degradation?</p> <p>For many years, <i>Bacteroides</i> spp. have been considered as unable to degrade inulin-type fructans. Recently, it has been shown that abundant <i>Bacteroides</i>, including <i>Bacteroides thetaiotaomicron</i>, are able to grow on oligofructose. However, the nature of the breakdown process seems different from bacteroidal starch degradation, as it is accompanied by the release of large quantities of monosaccharides in the extracellular environment. The ecological implications of colon bacteroidal fructan degradation are at present unknown.</p>

16.5 Conclusion

Due to its complexity, its proximity, and its close relatedness with human health, the human colon ecosystem has been fascinating microbiologists for many decades (Dethlefsen et al., 2006; Flint et al., 2008; Macfarlane and Cummings, 1991; Turrone et al., 2008; Zoetendal et al., 2006). Throughout the years, several

promising, innovative technologies have been introduced in the field of gut microbiology (Macfarlane and Macfarlane, 2004; Turnbaugh et al., 2007). Although many of these techniques have radically altered the dominating views on large-intestinal ecology, others have never fulfilled the expectations that accompanied them. Many of the latter, including molecular fingerprint approaches, have been presented as being able to abolish laborious and time-consuming microbiological methods involving plating, isolating, and subsequent identifying, that have been current practice in gut microbiology research from the early days on. However, these methods failed to comply with the three main necessities that still dominate human colon research: enumeration, identification, and functionality determination of bacterial species and groups.

Given the current lack of knowledge concerning the composition of the human colon microbiota, it is not surprising that the journey toward a functional understanding of the microbial colon community has only just begun. The recent isolation and cultivation of an ecologically crucial group of colon inhabitants, namely butyrate-producing large-intestinal bacteria (Barcenilla et al., 2000), leading to the identification of the source of colon butyrate (Pryde et al., 2002), has been a wake-up call for many gut microbiologists. Although the shallow diversity and wide-spread functional redundancy among colon bacteria makes it unnecessary to isolate every individual species and strain, it is now generally accepted that colon microbiology suffers from the lack of characterized representative species. Nowadays, a revival of the interest in the metabolic characterization of individual colon bacteria can be noted, especially regarding *Firmicutes* species (Duncan et al., 2007b). However, metabolic profiling is only the first step in niche identification. Areas including spatial distribution, bacteriocin production, bio-film formation, quorum sensing, and horizontal gene transfer remain presently largely unexplored (Flint et al., 2008; Macfarlane et al., 2000; Xu et al., 2007).

The Human Microbiome Project, a world-wide attempt to increase understandings of the microbial components of the human superorganism, offers some promising opportunities to research on microbial and ecological interactions within the gut microbiota. Although the project focuses on the relationship between the human host and the microbial community as a whole, the metagenomic approach lifts up the cover of the black box and facilitates the study of bacterial interactions (Handelsman, 2008; Turnbaugh et al., 2007). Moreover, in an early stage of the project, it includes an emphasis on isolation of previously uncultured gut bacteria, to extend current genome libraries. Indeed, a full understanding of the colon ecosystem will only emerge from combining information

from molecular tools for bacterial enumeration, monitoring of gene expression, and tracking of metabolites with physiological fermentation data obtained from (co)culturing representative gut isolates.

List of Abbreviations

- DP* Degree of polymerization
SCFA Short-chain fatty acids

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