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REVIEW ARTICLE

Microbial biotransformations in the human distal gut

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The human distal gut is home to a rich and dense microbial community with representatives of all three domains of life which are intricately connected with our physiology and health. The combined genomes of these microbes, collectively called the human microbiome, vastly expand the metabolic capacities of our own genome, allowing us to break down and extract energy from dietary compounds that human enzymes cannot digest. In addition, the variable composition of these communities and their biotransformations might explain inter-individual differences in toxicities, tolerances and efficacies for certain drugs. Recent advances in sequencing technologies and bioinformatics have provided exciting new insights into the genomes of our microbial symbionts, their functional capacities and the interactions between these microbes and their human host. This review summarizes the metabolic conversions of dietary components and pharmaceuticals that take place in the human distal gut, as well as their implications for human health.

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Abbreviations

HMP, Human Microbiome Project; SCFAs, short-chain fatty acids; spp., species; SRB, sulfate-reducing bacteria; TMA, trimethylamine; TMAO, trimethylamine-N-oxide

The human gut microbiome

The human gut is home to 100 trillion microorganisms, most of which live in the colon, the distal part of the gastrointestinal tract. Collectively, these microbes are called the human gut microbiota or, if also referring to their genomes and the surrounding habitat, the human gut microbiome. The vast majority of these microorganisms are bacterial, although archaeal and fungal species are part of the gut microbiota as well (Human Microbiome Project Consortium, 2012; Hoffmann *et al.*, 2013; Li *et al.*, 2014). In addition, the intestinal microbiome includes eukaryotic viruses and bacteriophages, and although their numbers and diversity are much less well studied and not part of the usual estimate of 100 trillion microbes, they are likely to be present in high numbers as well (Robinson and Pfeiffer, 2014). Other human body sites such as mouth and skin are also colonized with microbes, but their numbers and densities are considerably outnumbered by those living in the distal gastrointestinal tract (Sender *et al.*, 2016).

Symbiotic interactions, that is, close biological interactions between two or more different organisms, between multicellular eukaryotic life-forms and microbes, date back to ancient times and probably played important roles in plant and animal evolution. It has been proposed that the phenotypes and functional capacities of eukaryotic hosts cannot be viewed separately from that of their indigenous microbiotas, but that instead both host and their symbionts have to be viewed as a network of inter-genomic associations, or even a single biological unit (Bordenstein and Theis, 2015).

The main function of the mammalian gut microbiota is to assist with food digestion and energy extraction, by degrading dietary components such as fibre and cellulose that cannot be broken down and utilized by the host's functional capabilities alone. In addition, microbial colonization of the gut educates the host's immune system and helps to shape the correct development of anatomical structures in the intestinal tract (Round and Mazmanian, 2009). It has been estimated that the combined genomes of our gut microbiome encode for 500 times as many genes than the human genome

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(Li *et al.,* 2014), and it is thus not surprising that this microbial community is capable of many more biochemical conversions and reactions than its human host. Our gut microbes are basically small biochemical factories, expanding our body's metabolic capacities many times (Grice and Segre, 2012).

The gut microbiome is intricately connected to other organs and our general health. There is accumulating evidence for bidirectional communication between the gut microbes and our brain, in a concept called the gut-brain-axis, in which microbes might have an influence on the host's brain development and behaviour, potentially either directly through microbial metabolites in the blood stream, or indirectly by, for example, changing the expression of certain genes (Dinan and Cryan, 2017).

Recent advances in high-throughput DNA sequencing technologies have enabled us to assess not only the members of a microbial community by amplification of marker genes such as ribosomal RNA genes but also to analyse their full genomes and implied functional capabilities in much greater detail than before. The insights delivered by these sequencing surveys can now be complemented and expanded by other technologies such as transcriptomics, proteomics and metabolomics. Together, these novel 'omics' strategies have led to a much better understanding of the composition of the human-associated microbial communities, the metabolic reactions they perform and their interactions with the human host (Franzosa *et al.,* 2015).

Animal models, albeit artificial and not always an exact representative for human physiology, have made a considerable contribution to microbiome research as well (Kostic *et al.,* 2013; Sonnenburg and Bäckhed, 2016). The blood of mice colonized with a normal microbiota contains dozens of metabolites not present in the blood of germ-free mice, that is, animals delivered by Caesarean section and raised in sterile isolators, suggesting that the presence of a microbiome might have a large effect on the biochemistry of the host (Wikoff *et al.,* 2009).

Here, we will provide a broad overview of the main metabolic processes performed by the human gut microbiota, focusing on dietary and xenobiotic 'input' molecules and microbial output molecules that are relevant for the physiology of the host. Many excellent reviews have been written about specific subtopics (Flint *et al.,* 2012; Ursell and Knight, 2013; Koh *et al.,* 2016; Louis and Flint, 2016; Spanogiannopoulos *et al.,* 2016; Stilling *et al.,* 2016; Zhang and Davies, 2016), but we will provide a basic overview of prevalent commensal species and the main metabolic processes they perform.

Composition of the healthy intestinal microbiome

The human gut microbiome consists of hundreds of microbial species, most of which belong to two bacterial phyla: Bacteroidetes and Firmicutes. Together, these two bacterial taxa constitute the vast majority of the gut community in stool samples, irrespective of diet or geographical location (Human Microbiome Project Consortium, 2012; Obregon-Tito *et al.,* 2015; Falony *et al.,* 2016). Among the Bacteroidetes phylum, *Bacteroides* and *Prevotella* are the most abundant and prevalent genera (Table 1). Within Firmicutes, prevalence and abundance is highest for the *Blautia*, *Eubacterium*, *Faecalibacterium*, *Roseburia* and *Ruminococcus* genera (Falony *et al.,* 2016; Zhernakova *et al.,* 2016). Other less prominent but prevalent phyla are Actinobacteria, to which *Bifidobacterium* species belong, Fusobacteria (genus *Fusobacterium*) and Verrucomicrobia (genus *Akkermansia*). A novel phylum called Melainabacteria, related to Cyanobacteria, have recently been recognized as inhabitants of the human gut (Di Rienzi *et al.,* 2013).

The most commonly found representative of intestinal archaea is *Methanobrevibacter smithii*, which can be found in 25–95% of human stool samples (Hoffmann *et al.,* 2013). In addition to bacteria and archaea, eukaryotes such as fungi are commonly detected in stool samples as well. In a study of 96 healthy volunteers, fungi were found in all stool samples, with *Saccharomyces*, *Candida* and *Cladosporium* as the most prevalent (Hoffmann *et al.,* 2013).

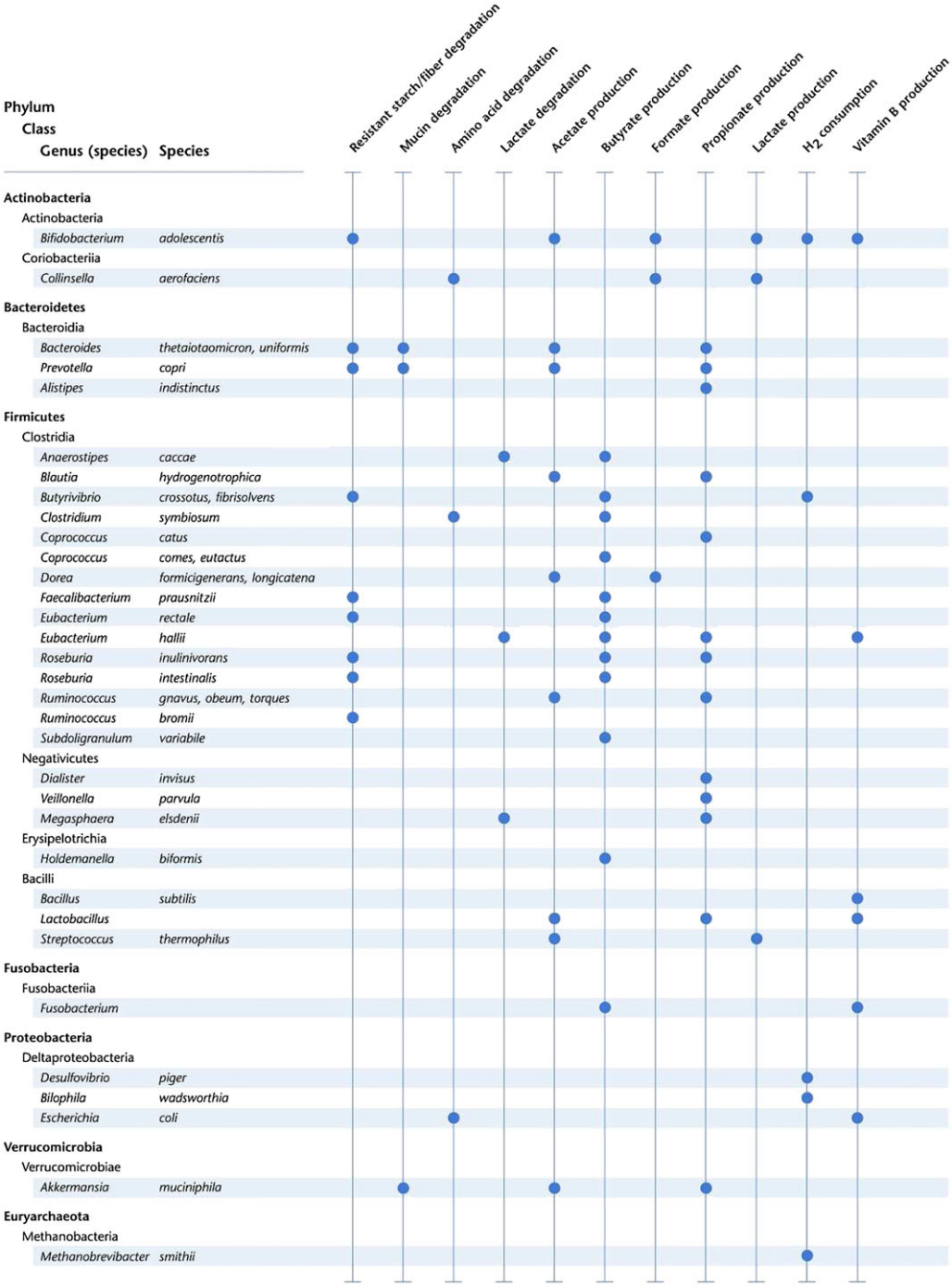
Despite a myriad of studies on the composition of the human microbiome of healthy controls and that of diseased patients, and the relative uniform prevalence of the same bacterial phyla among healthy populations, it has been surprisingly hard to define exactly what constitutes a healthy microbiome. Large inter-individual differences in microbial species composition and relative abundance among healthy subjects or populations from different geographical regions have made it challenging to identify the 'core' members of a healthy gut community (Lloyd-Price *et al.,* 2016).

Most large-scale gut microbiota studies have been performed on North American and European subjects (Qin *et al.,* 2010; Human Microbiome Project Consortium, 2012; Falony *et al.,* 2016; Zhernakova *et al.,* 2016). Even within these Western populations, it is hard to define a core microbiome. In a combined dataset of stool samples from nearly 4000 Belgian, Dutch, UK and US individuals, the core microbiome (taxa present in 95% of the samples) consisted of only 17 out of the 664 genera found (Falony *et al.,* 2016). Gut microbiomes from other parts of the world often have a very different composition from those from Western individuals (Yatsunenko *et al.,* 2012; Li *et al.,* 2014; Obregon-Tito *et al.,* 2015; Nishijima *et al.,* 2016), further narrowing this core (Falony *et al.,* 2016). Many of these microbiota differences can be explained by variations in dietary intake. For example, Western diets high in animal protein and fat and low in fibre are associated with a higher relative abundance of *Bacteroides*, while *Prevotella* is more abundant in people consuming plant-based, fibre-rich diets (Yatsunenko *et al.,* 2012; Obregon-Tito *et al.,* 2015).

Although it is hard to define the microbial taxa that constitute the healthy human microbiome, such worldwide studies have brought many new insights into the factors that determine its membership. The gut microbiome composition is believed to be the result of a combination of stochastic, lifestyle and host genetic variation. Diet, medicine use, age, health status and stool consistency were found to be the strongest environmental factors shaping the human gut microbiome (Falony *et al.,* 2016; Zhernakova *et al.,* 2016), while variations in the host genome play an important role as well (Blekhman *et al.,* 2015; Goodrich *et al.,* 2016).

Table 1

Abundant intestinal microbiota members and their metabolic conversions



The data shown here are compiled of data provided in a number of reviews (Louis and Flint, 2016; Magnúsdóttir *et al.*, 2017; Desai *et al.*, 2016; Koh *et al.*, 2016; Ze *et al.*, 2015; Blekhman *et al.*, 2015; Reichardt *et al.*, 2014; LeBlanc *et al.*, 2013; Flint *et al.*, 2012; Nakamura *et al.*, 2010; Belenguer *et al.*, 2006). Most Bacteroidetes and Firmicutes species are capable of producing vitamin B8, as reported by Magnúsdóttir *et al.* (2017). Genus and species names shown in bold were reported to have an abundance of over 1% in a cohort of 1135 Dutch individuals (Zhernakova *et al.*, 2016)

Microbiome composition not only varies between individuals but also over time. A person's individual gut microbiome composition appears to be relatively stable in the absence of disease and dietary or lifestyle changes but can quickly and dramatically respond to an altered diet, international travel, food poisoning (David *et al.*, 2014a,b) or antibiotic use (Dethlefsen and Relman, 2011). The fast response of the gut microbiome to such changes is thought

to be the result of the rapid growth rate of many bacterial species and the regular large expulsion of gut contents (Sonnenburg and Bäckhed, 2016). In addition to these rapid temporal responses, age has been shown to affect the composition of the gut microbiome as well, albeit at a much slower rate (O'Toole and Jeffery, 2015).

Despite these temporal and population differences, patterns of gut microbial composition in health are starting to emerge. A dataset obtained from a healthy subset of nearly 1000 subjects from the uBiome citizen science cohort was used to define normal ranges of 28 microbial taxa (Almonacid *et al.,* 2017).

Main functions of the gut microbiome

While human enzymes in the small intestine break down most dietary ingredients such as proteins, starch and fatty acids into absorbable smaller molecules, such as amino acids, and monosaccharides, not all molecules present in the diet can be digested in this part of the gastrointestinal tract. The human genome does not encode for enzymes that break down complex proteins and complex carbohydrates, that is, fibres and other plant-derived polysaccharides. These molecules will therefore reach the colon largely intact, where they can be digested by the gut microbiome (Flint *et al.,* 2012). In addition, the ability to degrade complex carbohydrates can be driven by the diet of the human population.

The most important function of the human microbiome in the distal gut is to extract energy from these otherwise indigestible dietary components (Flint *et al.,* 2012). Not surprisingly, many metabolic processes in the colon lumen are dedicated to this task. Reconstructing the metabolic pathways of the different body sites sampled in the Human Microbiome Project (HMP) consortium showed site-specific metabolic profiles (Human Microbiome Project Consortium, 2012). Human gut metabolic profiles as determined by metagenomic sequencing were characterized by glycosaminoglycan degradation, which was rare or absent in profiles from other body sites. This functionality was remarkably similar within gut samples from all HMP individuals despite large inter-individual variations in microbial species composition (Lozupone *et al.,* 2012) emphasizing that the composition of the gut microbiome is not as much about 'Who is there?', but about 'What are they doing?' This metabolic functional redundancy is likely to confer stability and resilience to the gut microbiota in the setting of dietary and environmental disturbances (Moya and Ferrer, 2016). Although inter-individual metabolic capabilities are very similar overall, there are also studies showing population-specific variations. Of note, *Bacteroides plebeius* strains from Japanese subjects harbour genes encoding for porphyranases and agarose (Hehemann *et al.,* 2010). These genes are absent in other populations and are thought to have been acquired by *B. plebeius* by horizontal gene transfer from marine bacteria through the consumption of seaweed, thus highlighting the role of the environment as a selective force on the functional potential of the human microbiota.

In addition to the degradation of polysaccharides, other important functions of the gut microbiome include the synthesis of short-chain fatty acids (SCFAs), specific lipopolysaccharides and certain vitamins and amino acids (Lloyd-Price *et al.,* 2016). A recent metabolic genome reconstruction of 773 members of the human gut microbiome genomes found genes encoding for 3200 unique chemical reactions, suggesting that this community encodes for hundreds or even thousands of metabolic pathways (Magnúsdóttir *et al.,* 2017).

Metabolism of human milk in infants

The microbiome and metabolic reactions of the human infant gut are distinct from those of the adult gut. Colonization of the infant gut starts immediately after birth, in a process that is thought to involve initial seeding with vaginal and skin microbes derived from the mother, which during the first months of life are gradually replaced with strains derived from other sources, with larger shifts in microbial composition around the time of weaning or around antibiotic treatment (reviewed in Mueller *et al.,* 2015).

Human milk is exceptionally rich in lactose, fatty acids and hundreds of different types of oligosaccharides consisting of different combinations of sugar moieties connected through a variety of glycosidic bonds, some of which are sialylated (Smilowitz *et al.,* 2014). Like dietary fibres in the adult gastrointestinal tract, the milk oligosaccharide glycoside and other bonds cannot be lysed by human genome-encoded enzymes, and the infant relies on bacteria to digest these compounds. The microbes needed to digest them are thought to be vertically transmitted from mother to infant through the milk (Mueller *et al.,* 2015). These bacteria, in particular *Bifidobacterium infantis*, *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, are abundant in the gut microbiota of most exclusively breastfed infants in the first months of life (Yatsunenko *et al.,* 2012; Bäckhed *et al.,* 2015). The genomes of these species are well equipped for the digestion of the oligosaccharides present in human milk, encoding for several receptors, intracellular and extracellular glycoside hydrolases and sialidases that can digest the many sugar components of human milk oligosaccharides (Sela *et al.,* 2011).

When breastfeeding stops and solid foods are introduced, the infant gut microbiota starts a trajectory towards a more adult-like composition characterized by an increase in the abundance of *Bacteroides*, *Clostridium*, *Faecalibacterium* and *Ruminococcus* (Bäckhed *et al.,* 2015; Mueller *et al.,* 2015). The composition of the infant gut microbiota continues to increasingly resemble that of an adult until it reaches maturation at 3–4 years of age (Yatsunenko *et al.,* 2012).

Microbial fermentation in the adult distal gut

In the adult gut, undigested dietary fibre, carbohydrates and proteins are fermented and further metabolized by the microbial communities in the caecum and colon (Figure 1). The main end products of the fermentation of complex carbohydrates are SCFAs, such as **acetate**, **propionate** and **butyrate**, and gases, such as CO_2, **H_2S** and NH_3. SCFAs are volatile fatty acids with one to six carbon atoms in straight or branched-chain conformations (den Besten *et al.,* 2013;

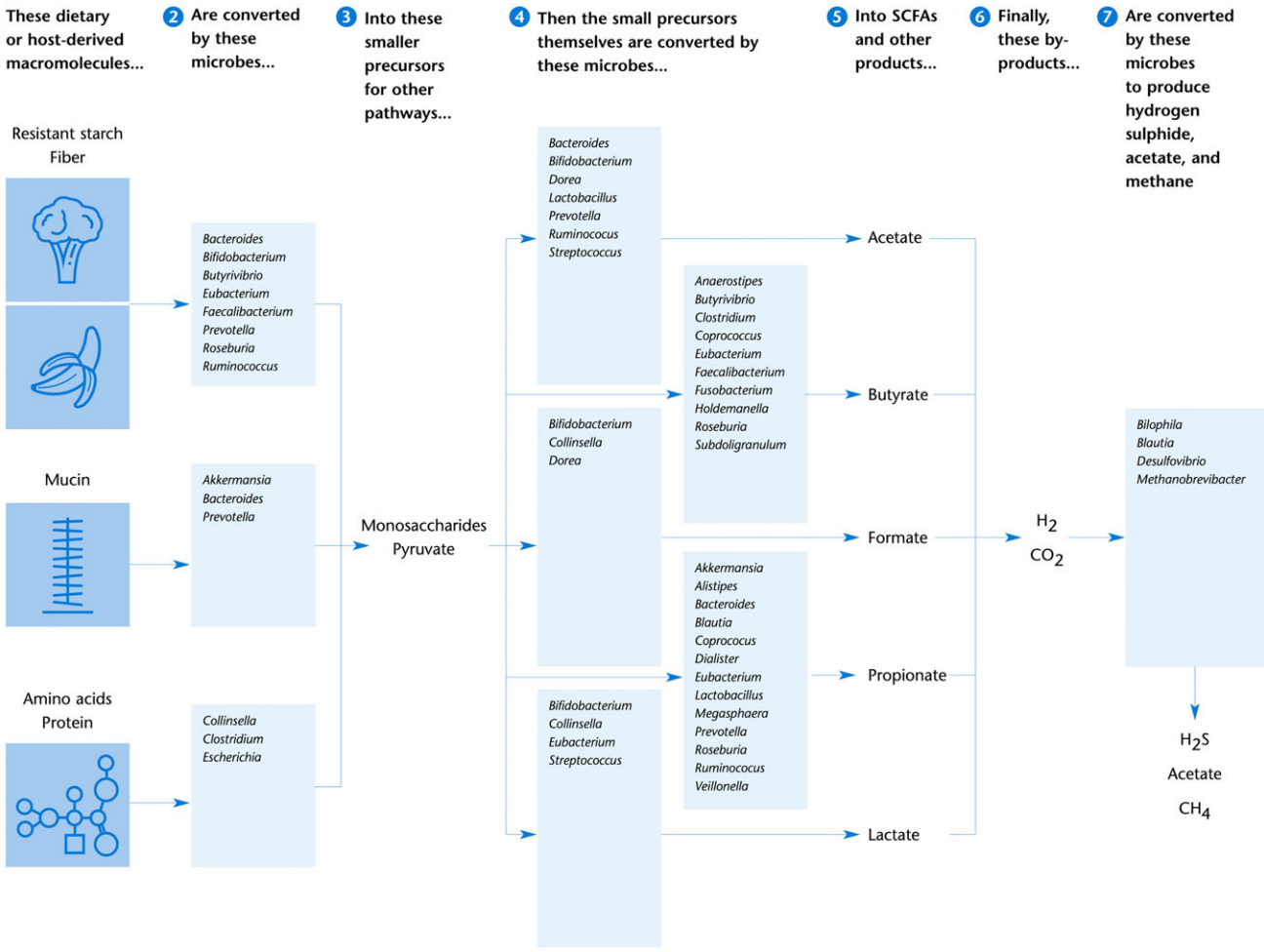


Figure 1

Main microbial fermentation pathways in the human gut. Boxes show the bacterial and archaeal genera involved in the digestion of macromolecules and the generation of SCFAs and other small molecules. The main species within those genera performing these reactions within the human gut are shown in Table 1. This graph is a simplified scheme; not all conversions and cross-feedings could be shown here. For example, acetate and lactate can be used by some gut bacteria as a precursor to produce butyrate. Compiled from data provided by Louis and Flint (2016); Magnúsdóttir *et al.* (2017); Desai *et al.* (2016); Koh *et al.* (2016); Ríos-Covián *et al.* (2016); Ze *et al.* (2015); Blekhman *et al.* (2015); Reichardt *et al.* (2014); LeBlanc *et al.* (2013); Flint *et al.* (2012); Nakamura *et al.* (2010); Belenguer *et al.* (2006).

Koh *et al.*, 2016; Ríos-Covián *et al.*, 2016). In the human colon, acetate (C2), propionate (C3) and butyrate (C4) are the most abundant, collectively accounting for over 90% of SCFAs (Ríos-Covián *et al.*, 2016). These three compounds are present in the ratio 60:20:20 respectively (den Besten *et al.*, 2013).

Fermentation starts with the digestion of plant-derived complex polysaccharides that were not digested by human enzymes in the small intestine. These dietary fibres include glycans such as cellulose, pectin and amylose, which consist of polymers of monosaccharide units. Glycoside hydrolases, the enzymes that can break down the bonds connecting these polymers, are found in specific distal gut microorganisms such as *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Prevotella*, *Roseburia* and *Ruminococcus* spp. (Table 1) (Flint *et al.*, 2012). The bacterial species that have this functionality are well equipped for this task. The genome of *B. thetaiotaomicron* in particular contains 172 glycosyl hydrolases and 20 sugar-specific transporters (Xu *et al.*, 2003). *Ruminococcus bromii*, another abundant member of the human gut microbiome, has a specialized genome with 21 glycoside hydrolases (Ze *et al.*, 2015). The genomes of *Bifidobacterium* species contain a large number of carbohydrate-modifying enzymes as well (Pokusaeva *et al.*, 2011). Regardless of bacterial species or enzymes, the breakdown of dietary polysaccharides leads to the generation of **pyruvate**, an important metabolic intermediate that forms the starting point for numerous anabolic pathways, many of which lead to the synthesis of SCFAs (Koh *et al.*, 2016; Stilling *et al.*, 2016).

Acetate is the most abundant SCFA in the distal gut. It is generated by gut microbes through two different metabolic routes. About two-thirds of the acetate is produced from pyruvate as the result of complex carbohydrate fermentation, and this capacity is present in a wide range of enteric bacteria (Ríos-Covián *et al.*, 2016). The remaining acetate is made by

acetogenic bacteria such as *Blautia* spp., which synthesize it from hydrogen (or **formate**) and carbon dioxide (see below) (Nakamura *et al.,* 2010; den Besten *et al.,* 2013; Koh *et al.,* 2016; Ríos-Covián *et al.,* 2016).

Propionate formation from dietary carbohydrates and amino acids by gut bacteria mainly occurs through two pathways, the **succinate** pathway or the propanediol pathway (Louis and Flint, 2016). The succinate pathway is present in many Firmicutes/Negativicutes (including *Veillonella*, *Dialister* and *Megasphaera* spp.), in Bacteroidetes (*Bacteroides*, *Prevotella* and *Alistipes*) and in Verrucomicrobia (*Akkermansia*), and this is likely to be the most important route in the human gut (Reichardt *et al.,* 2014). The propanediol pathway is found in Proteobacteria and *Ruminococcus* spp. (Reichardt *et al.,* 2014).

Butyrate biosynthesis pathways have been found in several phylogenetic groups of gut bacteria and all involve the conversion of butyryl-CoA to butyrate (Stilling *et al.,* 2016). The majority of the gut butyrate producers do this *via* butyryl-CoA:acetate CoA-transferase; this pathway is present in, for example, *Anaerostipes* spp., *Eubacterium* spp., *Faecalibacterium prausnitzii* and *Roseburia* spp. Alternatively, butyrate can be synthesized *via* phophotransbutyrylase and butyrate kinase in the genera *Butyrivibrio*, *Coprococcus* and *Subdoligranulum* (den Besten *et al.,* 2013; Louis and Flint, 2016; Ríos-Covián *et al.,* 2016).

Several gut bacteria produce **lactate**, such as *Bifidobacterium*, *Eubacterium*, *Lactobacillus* and *Streptococcus* spp. Most of this lactate is quickly converted into butyrate by other bacteria including *Eubacterium hallii* and *Anaerostipes caccae*, so its concentration in human faeces is usually very low (Belenguer *et al.,* 2006).

Like lactate, formate is present in low concentrations in the stool because it gets rapidly metabolized by other microbial species. Formate is produced by, for example, *Bifidobacterium*, *Collinsella* and *Dorea* spp, and can be used by *Desulfovibrio* and *Methanobrevibacter* spp. (Nakamura *et al.,* 2010; Rey *et al.,* 2013; Zhang and Davies, 2016).

When dietary fibre content is low, intestinal microbes will use less favourable nutrient sources such as dietary fats or proteins or even switch to digesting host-secreted mucins (Desai *et al.,* 2016; Koh *et al.,* 2016). For example, *Akkermansia muciniphila* ferments host mucins to form propionate (Derrien *et al.,* 2016). Digestion of host mucus glycoproteins can lead to erosion of the colonic mucus barrier, which in turn can lead to increased intestinal permeability and increased vulnerability for infections with pathogens (Desai *et al.,* 2016).

SCFAs function in human physiology

SCFAs produced by the gut microbiota are important molecules that can exert a wide range of functions (Figure 2). The main functions of intestinal SCFAs are to serve as an energy source for intestinal cells, signalling molecules, modulators of water and electrolyte absorption and regulators of lipid metabolism and components of the intestinal immune system (reviewed in, e.g. den Besten *et al.,* 2013; Koh *et al.,* 2016; Louis and Flint, 2016; Ríos-Covián *et al.,* 2016; Stilling *et al.,* 2016). In addition, SCFAs also modulate electrolyte and water absorption that can have various effects on organs beyond the gut. Therefore, SCFAs are key molecules in the communication between the host and microbiome, and they are believed to play important roles in health (Ríos-Covián *et al.,* 2016; Stilling *et al.,* 2016).

Clearly, SCFAs have many different functions, and the synthesis of these molecules is generally considered to beneficial for human physiology. Since SCFAs are the end products of microbial fibre degradation, their concentrations and ratios are closely associated with dietary fibre intake. Higher levels of SCFAs, in particular butyrate, and the bacteria that synthesize them are associated with reduced risk for various diseases, such as inflammatory bowel diseases, diabetes and intestinal cancer (Koh *et al.,* 2016; Ríos-Covián *et al.,* 2016).

In addition, butyrate and the other SCFAs might play a role in communication between the distal gut and more distant parts of the human body. SCFAs are detectable in peripheral blood, and it has been speculated that these small microbial molecules could even reach the brain. Here, they might exert effects on human mental state and behaviour, either directly or *via* other molecules, and as such could be the missing link in the gut/brain axis (Stilling *et al.,* 2016).

The relative low amounts of fibre in Western diets as compared to diets of, for example, hunter-gatherers, are likely to be associated with lower amounts and different types of SCFA. Although *in vivo* production and absorption of SCFA in humans are hard to measure, model systems have confirmed that caecal SCFA production is strongly dependent on dietary fibre content, and this might have important implications on human health (den Besten *et al.,* 2013).

Hydrogen production and conversion

One of the byproducts formed during the anaerobic degradation of organic matter is hydrogen (H_2) which is generated to dispose of reducing equivalents (Nakamura *et al.,* 2010). If the concentration of excess hydrogen in the colon reaches high levels, the fermentation pathways will be inhibited. Thus, the presence of hydrogen-consuming (hydrogenotrophic) microbes is needed to increase the efficiency of the fermentation process. There are several H_2-consuming members of the human gut microbiome, which can be categorized into three broad groups: acetogens, methanogens and sulfate/sulfite reducers (Nakamura *et al.,* 2010; Rey *et al.,* 2013).

Acetogens convert hydrogen into acetate by using CO_2 as an electron acceptor. These include *Blautia hydrogenotrophica*, which synthesize acetate from hydrogen (or formate) and carbon dioxide *via* the Wood-Ljungdahl pathway (Nakamura *et al.,* 2010; den Besten *et al.,* 2013; Koh *et al.,* 2016; Ríos-Covián *et al.,* 2016). As mentioned above, this acetate-generation pathway is different from the routes being used by a wide range of intestinal bacteria to directly ferment plant polysaccharides into acetate.

Methanogenesis, the conversion of hydrogen to methane, is a chemical process exclusively found in archaea, in which CO_2 is reduced using hydrogen or formate as an electron donor. In the human gut, this reaction is predominantly performed by *Methanibrevibacter smithii* (Nakamura *et al.,* 2010).

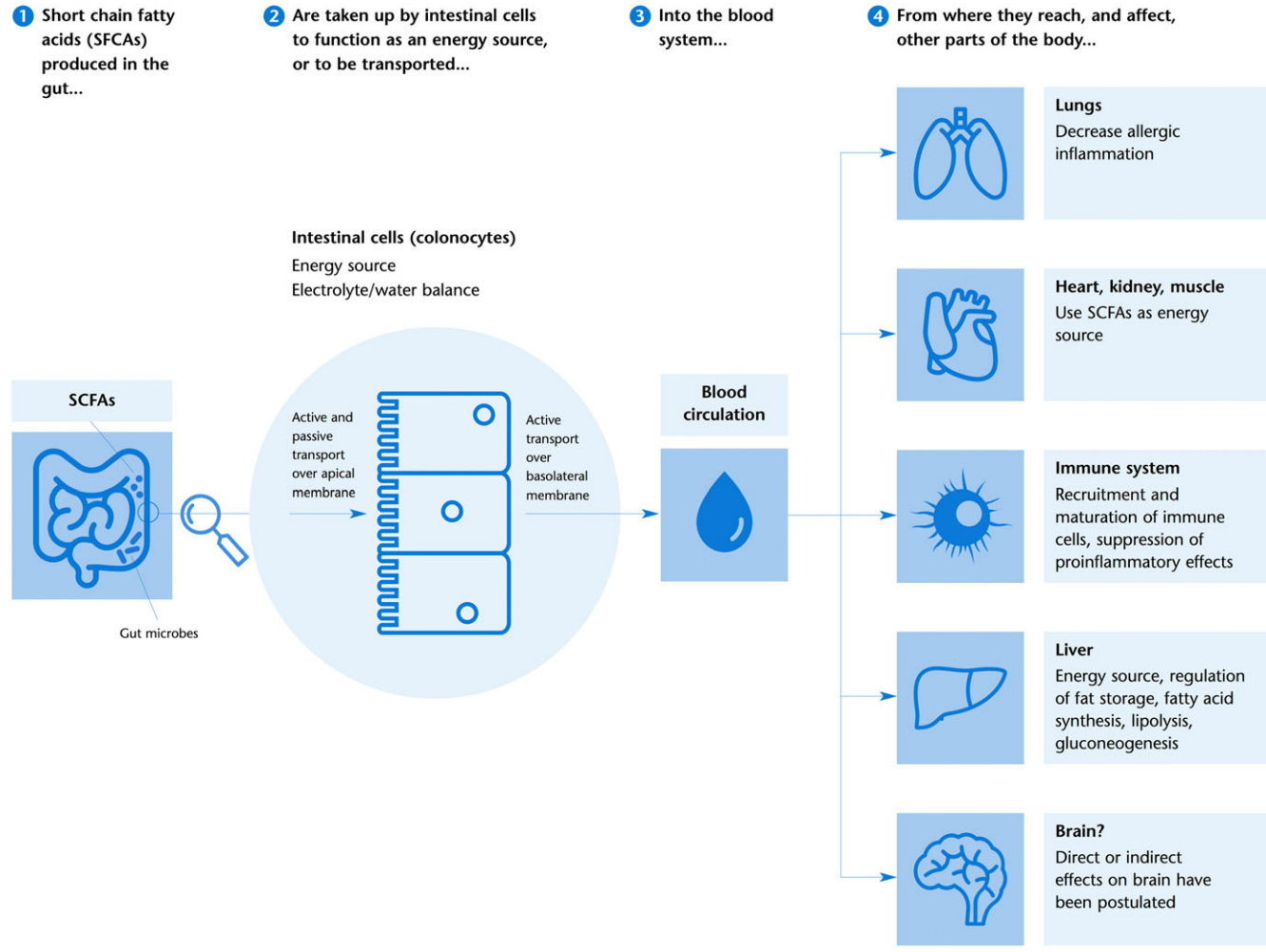


Figure 2

SCFAs effects on the gut and beyond. SCFAs are produced as the result of microbial fermentation in the distal gut (Figure 1) and absorbed by colonocytes through active and passive transport over the apical membrane. SCFAs are partly consumed by colonocytes as an energy source, while the remaining molecules are actively transported over the basolateral membrane and enter the blood circulation. From there, SCFA can affect processes in several peripheral organs by changing DNA transcription through the inhibition of histone deacetylation, binding to and activating GPCRs, or as metabolites in mitochondrial β-oxidation. Effects of SCFAs, in particular butyrate, on the brain have been hypothesized, either directly by passing the blood–brain barrier or indirectly by effects on the peripheral nervous system. Graphic based on den Besten *et al.* (2013), Koh *et al.* (2016) and Stilling *et al.* (2016).

Hydrogen can be also converted to H_2S by sulfate-reducing bacteria (SRB), which use sulfate as the electron acceptor. SRB in the human gut are almost exclusively *Desulfovibrio* species, which are present in the colon of about one-quarter of healthy US and European adults, with *Desulfovibrio piger* as their most common representative (Rey *et al.*, 2013; Zhernakova *et al.*, 2016). In the gut, the sulfate needed for this reaction is present in host mucins and in dietary components, since sulfate is used as an antioxidant in several food items (bread, dried fruit) or is used as a dietary supplement in the form of chondroitin sulfate. *D. piger* itself does not contain sulfatase genes to liberate the sulfate needed for the sulfate reduction and therefore relies on *B. thetaiotaomicron*, which produces sulfatases that release sulfate from host mucins or from chondroitin sulfate (Rey *et al.*, 2013).

In the complex microbial environment of the human colon, the efficient removal of the hydrogen generated during the fermentation of plant polysaccharides is essential for continuous SCFA production. The presence of both hydrogen-producers and hydrogen-consumers is one of the many syntrophic (cross-feeding) metabolic interactions found in the human gut, with sulfate reduction and methanogenesis the most commonly encountered pathways of hydrogen clearance in the colon (Ríos-Covián *et al.*, 2016; Stilling *et al.*, 2016).

Vitamin synthesis

Another important function of the gut microbiota is the synthesis of essential nutrients such as amino acids and

vitamins. These molecules are needed by the human body as a precursor for the synthesis of several enzymes or other compounds but cannot be made by humans themselves. Humans are therefore dependent on the presence of these essential molecules in our diet or supplements or on the capacity of our gut microbiome to synthesize them. Vitamins produced by the gut microbiome include **vitamin K** and most of the B-vitamins (LeBlanc *et al.,* 2013).

B-vitamins are a group of molecules including **biotin** (vitamin H), **folate** (vitamin B9), **thiamin** (vitamin B1) and cobalamin (vitamin B12) that are required in nucleotide and amino acid synthesis and metabolic processes. Most of these can be synthesized by members of the gut microbiota (LeBlanc *et al.,* 2013). In a systematic search for the presence of biosynthesis pathways for eight B-vitamins in the genomes of 256 common gut bacteria, about half of these genomes contained genes encoding the synthesis of at least one of these vitamins (Magnúsdóttir *et al.,* 2015).

Vitamin K (menaquinone) can be produced by the gut microbiota as well. Genes encoding vitamin K biosynthesis pathways were recently found to be present in the genomes of 118 out of 254 gut bacteria, including *Akkermansia*, *Bacteroides*, *Lactobacillus* and *Prevotella* spp., but whether these bacteria actually produce vitamin K remains to be determined (Ravcheev and Thiele, 2016).

Additional conversions in the human colon

Other than the well-studied metabolites discussed above, the human gut microbiome generates hundreds of other products, and many of these are thought to play roles in microbial–microbial or microbial–host interactions (Donia and Fischbach, 2015). Although the function of most of these molecules remains unknown as of now, ongoing research will probably uncover many new metabolites with interesting diagnostic and therapeutic applications. Here, we will list some recent discoveries in this field as a foretaste of the exciting possibilities to come.

First, *Oxalobacter formigenes* is an **oxalate**-degrading anaerobic gut bacterium thought to protect against hyperoxaluria and kidney stones by decreasing the amount and absorption of oxalate in the gut (Siener *et al.,* 2013). Oxalate is found in edible plants such as rhubarb, parsley and spinach. In the body, it can combine with calcium to form small crystals or larger stones that can clog the kidney tubules. Although the exact mechanisms behind the association of *O. formigenes* presence and reduced calcium oxalate stone formation are not yet known, this finding will lead to future kidney stone prevention and treatment strategies (Siener *et al.,* 2013).

Another example of a recent discovery is that genetic lactose-intolerance (i.e. a mutated gene encoding for the lactase enzyme) is associated with an increased abundance of *Bifidobacterium* spp. in stool. It was hypothesized that *Bifidobacterium* spp., which can metabolize lactose, allow a lactose-intolerant person to consume milk products (Blekhman *et al.,* 2015).

In contrast to the mostly beneficial gut microbial metabolites discussed above, other microbially synthesized molecules have been implicated in human disease. Dietary **L-carnitine** (abundant in red meat) and phosphatidylcholine are metabolized by gut microbiota members into **trimethylamine** (TMA). In the liver, TMA is converted into trimethylamine-N-oxide (TMAO), which is involved in atherosclerosis and is a risk factor for cardiovascular disease (Koeth *et al.,* 2013; Spanogiannopoulos *et al.,* 2016; Zhang and Davies, 2016). TMAO levels are higher in meat eaters than in vegetarians or vegans, and experiments in animals and human volunteers have suggested that the gut microbiota is likely to be responsible for the link between the consumption of red meat and cardiovascular disease (Koeth *et al.,* 2013). Recently, TMA conversion has been assigned to particular *Clostridia* and *Eubacterium* spp. (Rath *et al.,* 2017).

Drug metabolism

The vast combined array of chemical capabilities allows gut microbes to not only metabolize dietary and host components but also xenobiotics, that is, chemical substances that are not a natural part of an organism or its diet, such as drugs and pollutants. These biotransformations, most often reduction and hydrolysis, can result in three types of changes. They can activate drugs, inactivate drugs or make them more toxic. Currently, at least 50 different drug conversions performed by the human gut microbiota have been described and extensively reviewed (Ursell and Knight, 2013; Klaassen and Cui, 2015; Spanogiannopoulos *et al.,* 2016; Wilson and Nicholson, 2017). Some well-studied examples will be highlighted here briefly.

Certain medications, such as those with azo-bonds, rely on gut microbial enzymes to activate them. These drugs are administered to patients as pro-drugs, and microbial enzymes in the distal gut are needed to activate the compound into its effective form. Examples in this category include **sulfasalazine**, a drug to treat rheumatoid arthritis and inflammatory bowel diseases and the laxative pro-drug sodium picosulfate (Wilson and Nicholson, 2017).

In contrast, gut microbial enzymes can also inactivate certain drugs, making them less effective than anticipated. **Digoxin**, a drug used to treat congestive heart failure, can be inactivated by *Eggerthella lenta* strains that carry the *cgr* operon in their genome (Haiser *et al.,* 2013). For people who carry such *E. lenta* strains, the drug will not be as effective as for patients whose microbiomes contain *E. lenta* strains without the *cgr* operon or no *E. lenta* at all.

A third category of gut microbial conversions can make drugs more toxic or interfere with the host's detoxification process. **Acetaminophen** (paracetamol), a commonly used pain reliever and fever reducer worldwide, can induce severe hepatotoxicity when used in high amounts. However, acetaminophen toxicity has also been found in patients who consumed the drug at levels regarded as safe (Clayton *et al.,* 2009). This variable tolerance of individuals for acetaminophen is thought to be dependent on the composition of the gut microbiome. In the liver and intestinal mucosa, acetaminophen is detoxified by sulfonation or glucuronidation by human enzymes. However, certain gut

bacteria, such as *Clostridium difficile*, produce p-cresol from dietary protein residues. This p-cresol can be converted in the liver to p-cresol-sulfate, a process that competes with the detoxification of acetaminophen. Thus, individuals whose microbiota produces a lot of p-cresol are not as good at detoxifying acetaminophen as others, and the drug will be more toxic for them (Clayton *et al.,* 2009).

The chemotherapy prodrug **irinotecan**, mainly used to treat colon cancer, is activated by hydrolysis by host enzymes to **SN-38**, inactivated by glucuronidation in the liver and excreted in the bile. Expression of the inactivation enzyme can be influenced by genetic variations in the human genome or the gut microbiome composition. Bacterial β-glucuronidases in the colon can scavenge the glucuronic acid from the inactivated SN-38 molecule and re-activate the inactivated drug, which will lead to diarrhoea. Administration of irinotecan with selective bacterial glucuronidase-inhibitors was found to be very effective in mice (Roberts *et al.,* 2013).

Mycotoxins are poisonous small molecules produced by certain mould species that can be found as contaminants in food items such as peanuts, corn, spices and dried fruits. Deoxynivalenol is a toxin produced by *Fusarium* moulds and a frequent contaminant of cereals, often both in the toxic unconjugated form and a conjugated form. The gut microbiotas of some individuals hydrolyze the conjugated form to the toxic form, while those of other individuals can transform the toxic form into a less-toxic compound, thus leading to differential responses to contamination with this mycotoxin (Gratz *et al.,* 2013).

The examples listed above are just some of the many microbial conversions of xenobiotics described. Because of the large inter-individual variations in gut microbial composition, the same compound in the same dose can have a very different effect, with toxicity and efficacy varying from person to person. This microbial contribution in drug metabolism is often overlooked in clinical studies. Knowledge of the composition and metabolic activity of an individual's gut microbiota can be extremely helpful in predicting that individual's response to certain drugs (Ursell and Knight, 2013; Klaassen and Cui, 2015).

Concluding remarks

This review has provided a broad overview of known biochemical reactions performed by microbes in the human gut and their importance for human physiology. Some important concepts are worth restating here.

Firstly, gut microbial species do not exist as single entities but rather interact with each other by either competing for the same resources or by collaborating through metabolic cross-feeding, where one microbe's byproduct can be used as a substrate by another microbe (Flint *et al.,* 2012; Donia and Fischbach, 2015; Zhang and Davies, 2016).

Secondly, important functions of the human gut microbiome such as polysaccharide breakdown and SCFA synthesis are not performed by one particular phylogenetic lineage but by polyphyletic guilds of microbial taxa. This functional redundancy might provide the host with a robust microbiota, where the removal of one member does not necessarily result in the loss of an essential functionality. Therefore, it is generally accepted that a diverse gut microbiota, that is, one containing a high count of microbial species, is associated with health (Human Microbiome Project Consortium, 2012; Lozupone *et al.,* 2012).

Thirdly, the human gut microbiome might be responsible for some previously unexplained inter-individual responses to medications or dietary components and different disease risks. The composition of the human microbiome not only varies between individuals as a function of host genetics, geographical, societal and environmental factors but also over time with changes in diet, travel, disease status and medication intake (Human Microbiome Project Consortium, 2012; David *et al.,* 2014a,b; Blekhman *et al.,* 2015; Obregon-Tito *et al.,* 2015; Falony *et al.,* 2016; Zhernakova *et al.,* 2016). Alongside the enormous functional capacity of the intestinal microbiome, this adds a tremendous amount of inter-individual metabolic variation on top of that encoded by the human genome and an additional layer of intra-individual variation (Lloyd-Price *et al.,* 2016).

Finally, there are still many microbial biotransformations in the human gut that are poorly understood or remain to be characterized (Donia and Fischbach, 2015). Molecular surveys of the human gut have recently discovered novel lineages such as the Melainabacteria, which are probably capable of performing yet-uncharacterized metabolic routes (Di Rienzi *et al.,* 2013). Even for well-studied gut microbiome members such as *Escherichia coli* and *B. thetaiotaomicron*, many genes have not yet been assigned to a known function. In addition, genomic analysis is likely to reveal strain-level variations in metabolic capacities within species as well, as shown above for *E. lenta*. With the rapidly increasing amount of metagenomic data obtained from human gut samples, there is still a lot of knowledge to be gathered.

Such expected new insights of yet-to-be discovered metabolic functions and inter-individual differences are likely to greatly contribute to the personalized medicine field. In the near future, analysis of a patient's gut microbiome will be an integral part of personal clinical care and pharmaceutical development.

Nomenclature of ligands

Key ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.,* 2016).

Conflict of interest

All authors of this paper are employees of uBiome in San Francisco, CA, USA, and have received stock options as well as other compensation.

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