The human large intestine is colonized by a complex community of microorganisms, largely composed of strictly anaerobic bacteria with numerous physiological functions which impact on the host nutrition and health. Among these functions, the fermentation of substrates is of major importance for host health through the production of a wide variety of metabolites. The metabolic functions of the human gut microbiota are correlated with the nature of the substrates available for fermentation in the colon. These substrates are from exogenous (dietary fibers that are mainly plant polysaccharides) and endogenous (produced by the host and represent important source of nitrogen) sources. The metabolites produced from the microbial fermentation process in the gut are mainly absorbed and used by the host. Most of them have health benefits, but some may also have deleterious effects. The gut microbiota should thus be considered in relation to its environment, including dietary food and host factors. The interactions between food, intestinal microbiota and the host are fundamental to the maintenance of homeostasis in the ecosystem. Any disruption of this equilibrium could modify the functionality of the gut microbiota and lead to a pathological state.

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Introduction

The human large intestine is colonized by a dense and complex community of microorganisms, largely composed of strictly anaerobic bacteria. This intestinal microbiota exerts numerous physiological functions that have a significant impact on the host. Among these functions, the fermentation of substrates plays a key role in the maintenance of host health. The metabolic functions of the human gut microbiota are correlated with the nature of the substrates available for fermentation in the colon. The origins of these substrates are both exogenous (dietary fibers) and endogenous (produced by the host). A wide variety of substrates are thus available for fermentation by the intestinal microbiota, which contributes to the maintenance of the bacterial biodiversity of the ecosystem. Conversely, a variety of metabolites are produced from the microbial fermentation process, the main ones being short-chain fatty acids (acetate, propionate, butyrate), gases (hydrogen, carbon dioxide and, for some individuals, methane) and ammonia. The bioconversion of macromolecules by the gut microbiota into metabolites involves different functional groups of microorganisms (bacterial species sharing similar activity) with complementary activities. Although microbial diversity within the intestinal microbiota has been explored extensively in recent years, the functional aspects of this microbial diversity remain relatively unknown. The relationship between the phylogeny and the function of microorganisms is still difficult to establish, except for a few key metabolic characteristics such as butyrate production.

The fermentation metabolites produced by gut microorganisms are mostly absorbed and used by the host. Most of them have health benefits, but some may also have deleterious effects. Thus, interactions between food, intestinal microbiota and the host are fundamental to the maintenance of homeostasis in the ecosystem. Any disruption of this equilibrium could modify the functionality of the gut microbiota and lead to a pathological state.

This review focuses on two main metabolic functions of the intestinal microbiota: the metabolism of carbohydrates and of proteins.

Polysaccharide degradation and fermentation

The food-derived substrates are mainly composed of carbohydrates that have not been digested in the upper digestive tract [1]. They are mainly found in cereals, vegetables and fruits. These carbohydrates mainly consist of resistant starch, plant polysaccharides (plant cell wall and reserves) and some oligosaccharides and sugars such as inulin, gums, mucilages and fructooligosaccharides. The total amount of dietary polysaccharides that reach the colon daily varies from 10 to 40g depending on the diet [2].

The human gut ecosystem is well-adapted to utilizing the variety of polysaccharides present. The anaerobic degradation of these polysaccharides is a complex process involving several functional groups of microorganisms (Fig. 1). The different microorganisms interact to form a trophic chain, ensuring the conversion of macromolecules into short-chain fatty acids (SCFA) and gases. The first step of this chain consists of polysaccharide hydrolysis by hydrolytic bacteria, resulting in the release of smaller fractions. The products of sugar fermentation by hydrolytic and glycolytic microorganisms include intermediate metabolites such as formate, ethanol, succinate, lactate and hydrogen, which do not accumulate in the ecosystem but are further metabolized by other bacterial species into end products.
methane-status of the subject considered [9]. In contrast, high population levels (10⁹/g feces) of the xylan-degrading microbial community have been found in all subjects and are mainly composed of Bacteroides and Roseburia bacterial species [10]. The hydrolytic activity of Bacteroides sp. against xylan and soluble arabinogalactan has been widely studied [11], while Roseburia species have been isolated and characterized more recently. The degradation of plant polysaccharides involves a wide range of hydrolases (glycoside hydrolases, lysases, esterases). These enzymes often show complex multi-domain organization with multiple catalytic, structural and substrate-binding domains [4]. The genomic arrangement of these genes, as well as their regulation, further varies amongst species [4, 11]. Moreover, a bacterial species can produce different types of hydrolases. The degradation of complex structures such as plant cell walls thus requires the contribution of several bacterial species with complementary activities which interact, in particular through cross-feeding of sugars or hydrogen, to ensure efficient polysaccharide degradation [4, 12-14]. The growth of saccharolytic organisms is, to a large extent, controlled by the activity of the hydrolytic species and by competition between these different species for utilization of the released substrates.

Carbohydrate fermentation

Despite the wide variety of carbohydrates available and the large number of species able to ferment them, these substrates are catabolized by the intestinal microbiota according to a relatively limited number of metabolic pathways (Fig. 2). The majority of bacterial species use glycolysis, also known as the Embden-Meyerhof-Parnas pathway, to convert carbohydrates into pyruvate. Pyruvate is thus the central metabolite of these fermentation processes and is further converted through different pathways into end products of fermentation, which are the final electron acceptors. The main metabolites formed are acetate, propionate and butyrate. However, some bacterial species also produce intermediate metabolites such as succinate, lactate, acrylate, ethanol and formate, as well as H₂ and CO₂, which do not accumulate since they are quickly metabolized in situ by other bacterial species into major metabolites [15, 16]. The synthesis of these intermediate compounds also contributes to the maintenance of diversity within the colonic microbiota. The main SCFA (acetate, propionate and butyrate) are quickly absorbed by epithelial cells and are further metabolized in different organs (gut epithelium, liver, muscle, brain, etc.).
The majority of bacterial species present in the colon display *in vitro* mixed acid fermentation and therefore produce several metabolites from substrate utilization [15]. Acetate is synthesized during carbohydrate fermentation by the majority of the predominant species in the colon (*Bacteroides*, *Clostridium*, *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, etc.). The main pathway of acetate biosynthesis is the oxidative decarboxylation of pyruvate, leading to ATP synthesis (Fig. 2). Substantial utilization of acetate further occurs during butyrate formation [17].

Propionate is mainly synthesized by the predominant *Bacteroides* species, as well as by *Propionibacterium* and *Veillonella*. In the human colon, there are two possible pathways for propionate biosynthesis (succinate pathway and acrylate pathway) (Fig. 2). Formation of propionate through decarboxylation of succinate may be the major pathway used, particularly in predominant *Bacteroides* species, while the acrylate route from lactate is found in bacteria belonging to the clostridial cluster IX group [16, 17].

Butyrate-producing species in the colon have been rarely studied and identified until very recently [18], although this metabolite is well recognized for its health benefits. Pryde et al. [18] showed that the butyrate-producing community is composed of new species belonging to the genera *Eubacterium* and *Coprococcus*, as well as recently identified species belonging to the *E. rectale-Roseburia* and *Faecalibacterium* groups. These last bacterial groups are today considered to be predominant butyrate-producers in the gut [19-21]. The butyrate kinase pathway of butyrate biosynthesis was described first and involves condensation of two molecules of acetyl-CoA and synthesis of one molecule of ATP (Fig. 2). A second pathway employing a CoA-transferase that transfers the CoA moiety from butyryl-CoA onto acetate was recently found in the predominant butyrate-producers in the human gut [22, 23]. Acetate is a co-substrate of this reaction and it is hypothesized that the butyryl-CoA-CoA-transferase pathway may be promoted in the gut by the normally present high acetate concentration [17].

Metabolic cross-feeding between bacteria plays an important role in substrate fermentation in the gut ecosystem. For example, lactate does not accumulate in the colon of healthy individuals, although a significant proportion of intestinal bacteria can produce this metabolite. The bacterial species that mainly produce lactate from sugar fermentation are commonly known as lactic acid bacteria. In the human colon, lactic acid bacteria belong primarily to the *Bifidobacterium* and *Lactobacillus* as well as *Streptococcus* and *Enterococcus* species. The pathway of lactate formation involves the oxidation of...
of pyruvate by lactate dehydrogenase (Fig. 2). Lactate, which is produced from sugar fermentation in the colon, is to a large extent reutilized in situ following a different pathway leading to propionate or butyrate synthesis. Recent in vitro studies investigating the conversion of lactate by human fecal microbiota showed that the main metabolic pathway used differed significantly amongst individuals [24, 25]. About 60% of the fecal microbiota studied mainly transformed lactate into butyrate, while 20% formed propionate and another 20% metabolized lactate into both butyrate and propionate. Bacterial species able to convert lactate into butyrate were recently identified and include the species *Eubacterium hallii* and *Anaerostipes caccae* [26]. Colonic bacterial species belonging to *Veillonella* and *Propionibacterium* are capable of in vitro metabolism of lactate into propionate via the succinate decarboxylation pathway. However, some species of *Clostridium* as well as *Megasphaera elsdenii* present in the gastrointestinal tract of herbivores use the acrylate pathway to metabolize lactate into propionate [27]. This propionate synthesis pathway may exist in the human colon, but the species involved remain unknown. Ultimately, lactate is also a favored co-substrate for sulphate-reducing bacteria (SRB), which forms acetate and sulphides [28]. The SRB species, *Desulfovibrio piger*, was recently shown to compete efficiently with the predominant butyrate-producing species, *E. hallii*, for lactate utilization [29].

**Metabolism of proteins**

Protein metabolism is quantitatively less significant than that of polysaccharides, especially in the proximal colon. The total quantity of nitrogen-rich components present in the colon is estimated to vary from 6 to 18 g per day, with 1 to 2 g coming from the ileum [2]. Thus, the main sources of nitrogen are the host-generated substrates, with some dietary proteins able to reach the colon, depending on the diet and the structure of the protein-containing food.

The endogenous substrates come from both the small intestine (pancreatic enzymes, bile secretions, desquamated epithelial cells, mucus, etc.) and the colonic epithelium (mucopolysaccharides, mucus etc.). Unlike carbohydrate fermentation, protein degradation in the colon generates many metabolites that are potentially toxic to the host (phenols, indoles, ammonia, amines, etc.). This biodegradation requires the contribution of different bacterial species with complementary activities (proteases, desaminases, decarboxylases, etc.) which interact to ensure protein breakdown and metabolism. Carbohydrate fermentation largely contributes to the decreased availability of toxic metabolites formed from proteolysis through the stimulation of bacterial proteosynthesis.

**Proteolysis and metabolism of peptides and amino acids**

Proteins and peptides are the main nitrogen sources in the colon. Gut bacteria thus hydrolyze these polymers in order to obtain carbon and nitrogen for their own use. Proteolysis is thus a fundamental process in the colon; the mechanisms that regulate this process are still poorly understood [30]. The structure and solubility of proteins as well as their transit time are probably important factors. Intraluminal pH also plays a role since proteases have an optimum pH close to neutral. Factors that influence colonic pH, such as the production of acids during carbohydrate fermentation, are thus able to modulate proteolytic activity within the ecosystem. Therefore, bacterial proteases would be particularly active in the distal colon where the pH is close to 7.0 [31].

Protein hydrolysis by proteolytic enzymes (proteases) results in the release of peptides (Fig. 3). A large number of bacterial species in the human colon have proteolytic activity (*Bacteroides*, *Clostridium*, *Propionibacterium*, *Fusobacterium*, *Streptococcus* and *Lactobacillus*) [30]. Peptides can be assimilated by different bacterial species, and their utilization is frequently accompanied by the excretion of amino acids that are not necessary for bacterial growth (Fig. 3). These amino acids then become potentially available for other bacterial species in the colon that do not digest peptides [31]. There are a large number of species in the colon can use amino acids. Among them, some species of *Veillonella*, *Clostridium* and *Eubacterium* use amino acids as their main source of energy, as these bacteria do not ferment carbohydrates. However, many saccharolytic species also use amino acids and peptides as a nitrogen source [30]. Fermentation of amino acids involves a variety of oxidation and reduction reactions, with diverse final electron acceptors (unsaturated fatty acids, other amino acids, H₂, etc). Deamination of amino acids, leading to the formation of SCFA and ammonia, appears to be the main pathway used by bacterial species in the colon [31]. Acetate, propionate and butyrate are the major metabolites produced. However, a variety of other compounds are also formed during amino acid metabolism, such as phenols, dicarboxylic acids, and branched-chain fatty acids (isobutyrate, 2-methylbutyrate, isovalerate). Branched-chain fatty acids could be considered as markers of proteolysis in the colon. The concentration of such metabolites increases significantly from the proximal colon to the distal colon [30]. Phenol and indole, released from the breakdown of aromatic amino acids by certain *Clostridium*, *Lactobacillus* and *Bifidobacterium* species, are absorbed and detoxified by the colonic mucosa and are then excreted in urine. However, an increase in phenol and indole formation has been found to be associated with different diseases in humans, particularly in the case of colon cancer [31].

**Ammonia**

The majority of ammonia produced in the colon comes from the deamination of amino acids, with urea providing a minor contribution to this production [30, 31] (Fig. 3). Ammonia formed by the intestinal microbiota is absorbed by the colonic mucosa and transported to the liver by the portal vein where it is converted into urea and then excreted in the urine. Ammonia is also the main source of nitrogen for a large number of bacterial species in the colon. Inside the bacterial cell, amino transferases synthesize amino acids, which the bacterium requires, through the transfer of ammonia on carbon-skeletons [30, 31]. Ammonia is a potentially toxic compound for the host. At low concentrations (5-10 mM), this metabolite can alter the morphology and intermediate metabolism of intestinal cells and increase DNA synthesis [31]. Ammonia may thus be involved in the mechanisms of colon cancer initiation.
Ammonia concentrations in the colon result from a balance between the deamination of amino acids by bacteria and the use of ammonia by the cells for their biosynthesis. Through the stimulation of protein synthesis, carbohydrate fermentation contributes to decreased ammonia concentrations in the colonic lumen, together with absorption by the mucosa.

**Conclusion**

The large phylogenetic and functional biodiversity of human intestinal microbiota is controlled by both dietary, environmental and host factors. Interactions between food, intestinal microbiota and the host may be mediated, largely through bacterial metabolites produced from fermentation processes in the gut. Our current knowledge of this functionality of gut microbiota remains restricted to the physiological and genetic studies of a few cultivated species. Opportunities to expand this knowledge are now available through genome sequencing of a wide range of cultivated organisms, in addition to metagenomic approaches.

**Conflicts of interests**

None.

**References**


