Metabolic Diversity of the Intestinal Microbiota: Implications for Health and Disease[1,2]

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Abstract

The bacteria colonizing the human intestinal tract exhibit a high phylogenetic diversity that reflects their immense metabolic potential. By virtue of their catalytic activity, the human gut micro-organisms have an impact on gastrointestinal function and host health. All dietary components that escape digestion in the small intestine are potential substrates of the bacteria in the colon. The bacterial conversion of carbohydrates, proteins and nonnutritive compounds such as polyphenolic substances leads to the formation of a large number of compounds that may have beneficial or adverse effects on human health. J. Nutr. 137: 751S–755S, 2007.

In addition to being the organ for nutrient absorption, the human digestive tract also harbors a diverse community of mostly anaerobic microorganisms. The conditions in the various sections of the gastrointestinal tract differ considerably. This is reflected by the uneven distribution of the bacterial cells, with low concentrations of bacteria in stomach and duodenum [up to 10^3 colony-forming units (cfu)[3] per milliliter (cfu $\times$ mL^{-1})], increasing concentrations in jejunum and ileum (10^4–10^8 cfu $\times$ mL^{-1}), and the highest concentration in the colon (10^9–10^{12} cfu $\times$ mL^{-1}). The composition of the intestinal microbiota is relatively simple in infants but becomes more complex with increasing age, reaching a high degree of complexity in adults. At the level of bacterial species or strains, there is a high degree of variability among human individuals. Diet is one of the major determinants for the persistence of a given bacterium in the gastrointestinal tract because the diet provides nutrients not only for the host but also for the bacteria therein. Nondigestible food components serve as sources of energy and carbon for the human gut bacteria. By influencing the composition and activity of the human gut microbiota, diet has an indirect effect on the gastrointestinal function of the host and thereby on health. Intestinal bacteria affect the maturation and the maintenance of the immune system, influence the cell proliferation, and contribute to the salvage of energy. Moreover, the intestinal microbiota has a large catalytic potential, which may lead to the formation of metabolites with beneficial or adverse health effects.

Phylogenetic diversity and metabolic diversity

Recent evidence indicates that the cultivable fecal bacteria represent only a fraction of the bacteria actually present in the gut. The application of culture-independent methods revealed that the diversity of the fecal gut microbiota is considerably higher than anticipated (1,2). The proportion of undescribed species varied from 30 to 90%. Some 16S rRNA sequences indicated the presence of undescribed species that are closely related to described bacteria, whereas others indicated the presence of new species or genera with little relatedness to known bacteria. How can this high diversity of the gut microbiota be explained? It can be assumed that the number of fermentable substrates available to the bacteria in the gastrointestinal tract is a major reason for the complexity of the microbial community in the intestine. Not only the substrates originating from the diet or the host but also the large number of intermediates formed during bacterial breakdown of indigestible dietary components contribute to the large variety of substrates. The main substrates available to the bacteria in the human colon are dietary carbohydrates that have escaped digestion in the small intestine (3). These include resistant starches, dietary fiber (cellulose, hemicellulose, pectin, inulin), and unabsorbed sugars and sugar alcohols. However, dietary protein and protein from pancreatic enzymes and gastrointestinal secretions also contribute to some extent to the supply of substrates to intestinal bacteria (4). Mucus produced by the host and sloughed epithelial cells are other potential substrates.

The fermentation of complex polymers such as polysaccharides and proteins in the colon requires the cooperative action of

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[3] Abbreviations used: cfu, colony-forming unit; DMA, *O*-demethylangolensin; SCFA, short-chain fatty acids; SRB, sulfate-reducing bacteria; UC, ulcerative colitis.

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different microbial population groups. The breakdown starts with the depolymerization of complex carbohydrates and proteins and gives rise to mono- and oligomeric compounds that can be broken down further to the short-chain fatty acids (SCFA) acetate, propionate, and butyrate as well as to carbon dioxide and molecular hydrogen. Lactic acid, ethanol, succinic acid, and formate are important intermediates that are also degraded to SCFA, CO_2, and H_2. Proteins are broken down to peptides and amino acids, whose fermentation also results in the formation of SCFA, CO_2, and H_2. In addition, branched-chain fatty acids, ammonia, hydrogen sulfide, amines, phenols, indoles, and mercaptanes are formed (4).

Microbial activities and their relevance to health and disease

Formation of short-chain fatty acids. The formation of SCFA is of major relevance to the host. The daily production of SCFA has been estimated to be in the range of 400 mmol (5). The bacterial formation of SCFA enables the host to salvage some of the energy contained in dietary fiber that would be lost otherwise. Various tissues in the body are able to oxidize SCFA for energy generation. Butyrate is the preferred fuel of the colon epithelial cells that derive 70% of their energy from the oxidation of butyrate (6).

Butyrate has been proposed to lower the risk of colon cancer. This notion is based on a number of observations including the ability of butyrate to inhibit the genotoxic activity of nitrosamides and hydrogen peroxide in human colon cells (7) and the potential of butyrate to induce apoptosis in human colonic tumor cell lines in a p53-independent pathway (8). It has also been proposed that butyrate plays a major role in chronic inflammation of the intestinal mucosa. The inability of the colonocytes to oxidize butyrate was considered as a possible reason. In accordance with this notion, colon epithelial cells from ulcerative colitis (UC) patients were reported to have a reduced capacity to oxidize SCFA (9). However, more recent work has shown that the reduced butyrate oxidation rate observed in patients with active UC is reversed during remission, suggesting that UC mucosa is not intrinsically altered in butyrate oxidation and making the inability to oxidize butyrate unlikely to be a primary defect in UC (10). Nevertheless, SCFA irrigation for 2 to 3 wk has been shown to improve the macroscopic and histological signs of inflammation resulting from diversion colitis, which develops after complete diversion of the fecal stream (11). It is conceivable that butyrate acts at least partially via its effect on mediators of inflammation. Butyrate was shown to decrease the expression of the proinflammatory cytokines tumor necrosis factor-α (TNF-α), TNF-β, interleukin-6 (IL-6), and IL-1β by inhibiting the activation of nuclear factor κB (NF-κB) in lamina propria mononuclear cells from patients with Crohn's disease (12). Similarly, butyrate inhibits NF-κB activation in lamina propria macrophages of patients with UC and results in a significant decrease in the disease activity index in these patients (13).

Protein and amino acid fermentation in the colon. Whereas the bacterial conversion of fermentable carbohydrates occurs primarily in the proximal colon, the fermentation of proteins occurs mainly in the distal colon (4). Some of the products resulting from amino acid fermentation have some relevance to health. Oxidative or reductive deamination of amino acids leads to the formation of ammonia, whereas decarboxylation results in the production of amines. High ammonia concentrations have been shown to act as tumor promoters (14): Rats were treated with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine to induce tumors and subsequently assigned to either the control group or to the ammonium acetate treatment group. Sixty-nine percent of the animals in the treatment group had 28 adenocarcinomas, 8 of which were invasive, whereas only 40% of control animals had 12 adenocarcinomas, none of which was invasive.

The bacterial degradation of the amino acids cysteine and methionine results in the formation of H_2S (present as HS^- at neutral pH). A diet rich in meat correlates with high fecal concentrations of sulfide (15). H_2S is toxic and was shown to inhibit butyrate oxidation in colonocytes (16). It was subsequently proposed that H_2S could play a role in UC (17). Sulfate-reducing bacteria (SRB) were identified as another major source of H_2S. SRB couple the oxidation of lactate, ethanol, succinate, and H_2 to the reduction of sulfate to sulfide. Foods may contain high levels of oxidized forms of sulfur such as sulfate, sulfite, bisulfite, and sulfur dioxide. These food additives as well as endogenous sulfated mucins are potential sources of bacterial sulfide formation (18). A variety of morphologically and nutritionally different SRB were isolated from human feces and found to belong to 5 different genera with *Desulfovibrio* being the dominant genus (19).

The decarboxylation of amino acids in the colon results in the formation of amines. Under acidic conditions or catalyzed by intestinal bacteria, the latter may react with nitrite to form *N*-nitroso compounds. Many oral and intestinal bacteria are capable of reducing nitrate to nitrite. However, it is not clear whether bacterial nitrite is a key agent in nitrosation. In any case, the gut microbiota have the capacity to catalyze the formation of nitrosamines from secondary amines and nitrite (20). Nitrosamines are highly carcinogenic.

The anaerobic fermentation of the aromatic amino acids tyrosine and tryptophan by colonic bacteria gives rise to phenols and indoles, respectively, which are eventually excreted in the urine (4). Urinary indoles and phenols are increased in response to a protein-rich diet (21). Phenols such as *p*-cresol have been proposed to act as procarcinogens in colon cancer (22). *N*-Nitrosation of dimethylamine by nitrite is enhanced by phenol, and the reaction of phenol with nitrite produces *p*-nitrosophenol and *p*-diazoquinone, both of which are cancerogenic (23).

Transformation of bile acids and xenobiotics. Bile acids are secreted as glycine, taurine, or sulfate conjugates. These undergo deconjugation followed by a dehydroxylation reaction. For example, the deconjugation of glycocholate leads to cholate, which in turn is transformed by bacterial 7α-dehydroxylase to deoxycholate, 1 of the so-called secondary bile acids. There are good indications that secondary bile acids may act as tumor promoters (24).

Dietary components, pharmaceuticals, and endogenous metabolites with hydrophobic properties are transported to the liver, where they undergo oxidation by the cytochrome P_{450} system, resulting in the corresponding hydroxyl compounds. The latter are subsequently conjugated with either glucuronic acid, sulfate, or glutathione to increase their water solubility and facilitate their urinary or biliary excretion. Compounds excreted in bile reach the intestinal tract, where they undergo deconjugation by intestinal microorganisms. A large variety of gut microorganisms possess hydrolytic enzymes such as β-glucuronidase and sulfatase (25). The deconjugated metabolites may be reabsorbed, entering the enterohepatic circulation.

Bacterial degradation of oxalate in the intestine. *Oxalobacter formigenes* is present in the human intestinal tract, where it lives at the expense of the transformation of oxalate to formate and carbon dioxide (26). It has been shown that the absence of

O. formigenes is a risk factor for the formation of calcium oxalate stones (urolithiasis) (27) and that calcium oxalate stone formers have a low rate of colonization with *O. formigenes* (28). Moreover, the oral uptake of *O. formigenes* by human volunteers reduces the urinary oxalate excretion and results in oxalate-degrading activity in feces (29).

Activation and inactivation of bioactive food components. The human diet contains a large variety of plant-derived nonnutritive substances including lignans and flavonoids, which belong to the large group of polyphenols. Following their uptake with the diet, these compounds may be absorbed to some extent, but they may also undergo transformation by intestinal bacteria. The biological properties of the resulting transformation products may differ considerably from those of the original compound. The bacterial transformation of a bioactive compound may result in the formation of an inactive compound. Alternatively, the activity may be enhanced as a result of bacterial transformation, and an originally inactive compound may be activated through bacterial conversion.

Isoflavonoids, which are found for instance in soy, beer, or clover, have been reported to have estrogenic properties. Together with lignans (see below), they are called phytoestrogens, and, based on their purported biological effects, they have been proposed to be chemoprotective agents. The plasma concentrations of the flavonoids daidzein and genistein appear to be highly influenced by diet. Whereas the plasma concentration of daidzein and genistein was reported to be 383 nmol/L in Japanese men, it was only 12.5 nmol/L in Finnish men (30). This can be related to the high consumption of soy and soy products in Japan. Isoflavones have been associated with a number of health effects (31,32). These include the prevention of hormone-related cancers such as breast cancer, atherosclerosis, and osteoporosis in the alleviation of menopausal symptoms. Their inhibitory effect on tyrosine-specific protein kinases and on topoisomerases I and II, which influence cell proliferation, has been proposed as a possible mechanism of their action (33,34). It has been shown that the isoflavone daidzein undergoes transformation by intestinal bacteria to form products with either reduced or enhanced biological activity. In the human gastrointestinal tract, daidzein may undergo bacterial transformation to equol, which has a higher biological activity than daidzein (35,36). It is interesting to note that approximately 1 of 3 individuals produces equol (37). Alternatively, daidzein may also be degraded to *O*-demethylangolensin (DMA) (38), which in contrast to daidzein or equol is biologically inactive. The organism(s) catalyzing the formation of equol are not yet known. *Eubacterium ramulus*, which has become known for its ability to metabolize a number of flavonoids including quercetin, apigenin, and naringenin (39,40), was shown to convert daidzein to DMA, which, however, is not degraded any further by this organism (41). In contrast, the degradation of genistein by *E. ramulus* continues beyond the intermediate 6′-hydroxy-DMA. The latter undergoes cleavage to 4-hydroxyphenyl-2-propionic acid and phloroglucinol (41).

Plant lignans, e.g., secoisolariciresinol diglucoside, are converted by human intestinal bacteria to the enterolignans enterodiol and enterolactone. Therefore, bacteria influence the possible beneficial health effects of these active estrogen-like compounds. Lignans have been reported to have estrogenic and antioxidant activities (42,43). They may act through their ability to bind to proteins or inhibit relevant enzymes (44–46). In rats, lignans have been shown to reduce mammary tumor growth at various stages of carcinogenesis (47). However, evidence from human studies for a cancer preventive effect is insufficient (48,49). There are good indications that lignans have protective effects against diabetes (50) and coronary heart disease by their hypocholesterolemic and antiatherogenic effects (51).

Recently, we have used culture-based and molecular approaches to investigate the diversity and occurrence of lignan-transforming intestinal bacteria. In contrast to the bacterial production of equol, which is observed in 30% of human individuals, the conversion of secoisolariciresinol to enterodiol and enterolactone is widely distributed among human subjects. Eleven phylogenetically and functionally distantly related bacterial species that catalyze the *O*-deglycosylation, *O*-demethylation, dehydroxylation, or dehydrogenation step of secoisolariciresinol diglucoside have been isolated from human feces or obtained from bacterial culture collections (52). Most of these species are members of dominant intestinal microbiota, as determined by most probable number and fluorescent in situ hybridization enumerations (53,54). They include strains of the genera *Bacteroides*, *Clostridium*, *Eggerthella*, *Eubacterium,* and *Ruminococcus*. The in vitro transformation of secoisolariciresinol diglucoside to enterolactone has been demonstrated using a defined mixed culture of bacteria (**Fig. 1**). The challenge is now to bring to the test the relevance of these in vitro findings using animal models and to see how changes in bacterial population levels in the intestinal tract may influence the bioavailability and health effects of lignans.

The conversion of dietary components by intestinal bacteria leads to the formation of a large variety of metabolites. These may have beneficial or adverse effects on human health. Future activities will be directed to influence the gut microbiota in a targeted way, ideally by enhancing beneficial effects and minimizing adverse effects. However, before we are able to do so, further work is required to understand in more detail the processes underlying the bacterial conversion of dietary components.

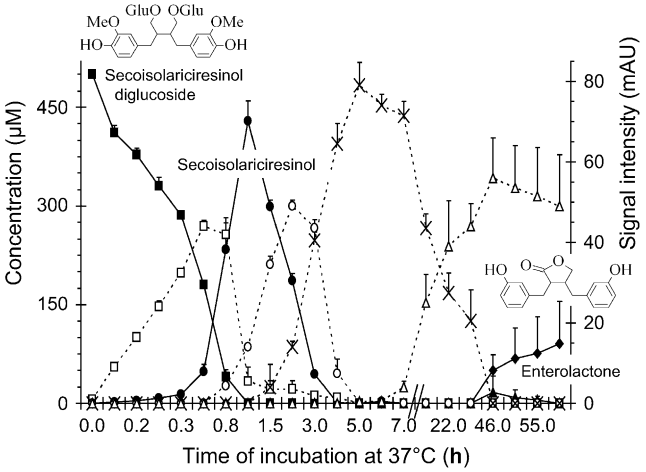

Figure 1 In vitro conversion of secoisolariciresinol diglucoside to enterolactone by a mixed culture of human intestinal bacteria. Four reactions must be catalyzed in sequence for the production of enterolactone. Bacterial strains were *Clostridium saccharogumia* DSM 17460^T (O-deglycosylation), *Ruminococcus productus* SECO-Mt75m3 (*O*-demethylation), *Eggerthella lenta* SECO-Mt75m2 (dehydroxylation), and *Lactonifactor longoviformis* DSM 17459^T (dehydrogenation). Lignan metabolites were detected by HPLC (53) at the times indicated. Dotted lines refer to lignan metabolites for which no standards were available; (□) secoisolariciresinol glucoside, (○) demethyl-secoisolariciresinol, (×) didemethyl-secoisolariciresinol, (△) 2,3-bis(3,4-dihydroxybenzyl)butyro-lactone. For these metabolites, the Y-values are the signal intensities measured at 285 nm by the UV diode array detector of the HPLC system. Resting cells were prepared as described previously (53) and incubated anaerobically under N_2/CO_2 (80/20; vol:vol). Two mixed cultures prepared from 2 independent batch cultures of each strain were analyzed. Values are means ± SD. The cell density of each strain in the mixed cultures was adjusted to ~10^9 cells/mL.

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