Pathways and Functions of Gut Microbiota Metabolism Impacting Host Physiology

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Abstract

The bacterial populations in the human intestine impact host physiological functions through their metabolic activity. In addition to performing essential catabolic and biotransformation functions, the gut microbiota produces bioactive small molecules that mediate interactions with the host and contribute to the neurohumoral axes connecting the intestine with other parts of the body. This review discusses recent progress in characterizing the metabolic products of the gut microbiota and their biological functions, focusing on studies that investigate the responsible bacterial pathways and cognate host receptors. Several key areas are highlighted for future development: context-based analysis targeting pathways; integration of analytical approaches; metabolic modeling; and synthetic systems for *in vivo* manipulation of microbiota functions. Prospectively, these developments could further our mechanistic understanding of host-microbiota interactions.

Keywords

Microbiota metabolites; pathways; receptor ligands; gnotobiotic mice; metagenomics; metabolomics; metabolic model

Introduction

Colonized at birth, the adult human gastrointestinal (GI) tract harbors $\sim 10^{14}$ bacteria belonging to at least several hundred species [1]. Collectively termed the gut microbiota, these bacterial populations impact an array of physiological functions in the GI tract, including digestion and immune response to foodborne pathogens. A well-known digestive function of the microbiota is the fermentation of complex carbohydrates to short chain fatty acids (SCFAs). Recent metabolomic studies have detected SCFAs and many other bioactive microbiota-derived metabolites in systemic circulation [2], supporting the view that these molecules constitute part of the neurohumoral communication axes that link the intestine with other organs such as the liver and brain.

Significant alteration in the microbial populations, or dysbiosis, correlates with not only GI diseases, but also other chronic diseases such as diabetes, cancer, and asthma. While it is difficult to establish causation, the case for dysbiosis as a contributing mechanism for some diseases has become increasingly compelling. Seminal work by the Gordon laboratory identified a pattern of dysbiosis in obesity that is characterized by a greater capacity for energy harvest [3]. Psychiatric disorders such as autism spectrum disorder (ASD) are frequently accompanied by changes to the intestinal microbiota composition [4,5]. Using a murine model of ASD, Hsiao et al. showed that alterations to the serum metabolite profile resulting from dysbiosis correlate with behavioral abnormalities; administration of a probiotic attenuated these abnormalities while reducing the serum levels of microbiota metabolites elevated in ASD mice [6].

These studies provide firm evidence that dysbiosis dramatically impacts host physiology; moreover, the functional consequences reflect alterations in the profile of microbiota-derived metabolic products. Many of these metabolites show activity as signaling molecules [7], and engage various host receptors and regulatory molecules *in vitro* and *in vivo* [8,9]; however, specific functions have been identified for only a small subset of these metabolites [10]. Aided by advances in sequencing and data analysis pipelines [11], the catalog of annotated genomes for bacteria found in humans [12,13] and model organisms [14] has rapidly expanded over the last several years. These developments present an exciting opportunity to integrate compositional data with measurements on the functional outputs of the microbiota to address fundamental questions regarding molecular mechanisms mediating host-microbiota interactions.

In this review, we discuss recent progress in characterizing the metabolic products of the gut microbiota and their biological functions in the context of host physiology. We focus on studies that investigate the responsible enzymatic pathways and bacterial groups. We also discuss representative methods and models, including metabolic models that could facilitate the integration of different types of data on microbiota composition and functional readouts.

Metabolic functions and outputs of the gut microbiota

Substrates metabolized by the gut microbiota include dietary residues, mucosal macromolecules (e.g. mucins), endogenous metabolites (notably bile acids), and xenobiotic chemicals. The major classes of dietary substrates (Fig. 1) comprise carbohydrates, amino acids, certain lipids (e.g., polyunsaturated fatty acids [15]), and phytochemicals [16].

Short-chain fatty acids (SCFAs)

The most abundant SCFAs in the intestine are acetate, propionate, and butyrate, which are primarily derived from carbohydrates [17]. Some fermentation of amino acids also occurs, generating alkyl carboxylic acids such as valerate and caproate [18]. Acetate and propionate are found throughout the small and large intestines, while butyrate is found mainly in the cecum and colon.

The main route for propionate synthesis proceeds through succinate, with methylmalonyl-CoA decarboxylase catalyzing a key step that results in the formation of propanoyl-CoA [19] (Fig. 2). An

alternative pathway involves hydrolysis of propane-1,2-diol into propanal. This pathway is present in several major phylogenetic groups, especially *Lachnospiraceae*, and likely plays an important role in fermenting deoxy sugar residues of host-derived glycans [19]. Butyrate production can also occur through several pathways, utilizing acetyl-CoA, glutarate, 4-aminobutyrate, or lysine as the major substrate (Fig. 2). A recent metagenomic analysis of stool samples from healthy human subjects found that the acetyl-CoA pathway is the most prevalent, followed by the lysine pathway [20]. The same study identified *Firmicutes* strains as the major butyrate producers, with the vast majority of these strains harboring genes for the acetyl-CoA pathway. Interestingly, the lysine and acetyl-CoA pathways often occur together, suggesting that the latter pathway could be cross-fed with protein degradation pathways, thereby providing adaptability to a protein-rich diet [20].

Utilized locally as an energy source by colonic epithelial cells, SCFAs are critical for maintaining intestinal barrier integrity by regulating expression of tight junction proteins. The best characterized host receptors for SCFAs are G protein-coupled receptors 41 (GPR41) and 43 (GPR43). GPR41 is widely expressed in a variety of tissues and cell types, and plays an important role in regulating whole body energy balance through its interaction with the gut microbiota [21]. GPR43 is most highly expressed in immune cells. Targeted gene knockout studies have shown that SCFAs activate cytokines and chemokines *in vitro* and *in vivo*, and that this response is GPR43 dependent [22].

Aromatic amino acid (AAA) derivatives

A major group of immunomodulatory microbiota metabolites derives from catabolism of AAAs (Fig. 3a). In both humans and mice, a principal route for tryptophan (TRP) catabolism is the kynurenine pathway. Depending on the cell type, the rate-limiting first step is catalyzed by tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase (IDO1). Kynurenine is an endogenous ligand for the arylhydrocarbon receptor (AhR), which regulates immune responses and inflammation through its effects on cytokine production and Treg cell development [23]. Because kynurenine production competes with other TRP pathways, IDO1 activity in immune cells of the intestine modulates the TRP pool available for microbiota metabolism [24]. Major products of TRP catabolism include indole-3-acetamide, tryptamine (TrA), and indole, formed via tryptophan 2-monooxygenase, tryptophan decarboxylase, and tryptophanase A (TNA), respectively [24]. These products can be further transformed into other indole-containing molecules such as indole-3-acetate (IAA) and indole-3-acetaldehyde (IAAld). The genes encoding the TRP reactions are found in many different phylogenetic groups of the gut microbiota [25] as well as in human and mouse genomes; of the aforementioned enzymes, only TNA is strictly bacterial. Nevertheless, metabolomic analyses of intestinal and fecal samples from GF and gnotobiotic mice indicate that the gut microbiota is a major source of these and other AAA derivatives (e.g. phenethylamine from phenylalanine and phenol from tyrosine) [2,25]. Like kynurenine, TrA, IAA, and IAAld are AhR ligands, and can modulate the host's immune reactivity [24] (Fig. 3b). Indole itself is a weak AhR agonist [26], but dose-dependently increases tight junction resistance of intestinal epithelial cells and attenuates indicators of inflammation [27], suggesting that microbiota-derived TRP metabolites may interact with host pathways through other receptors.

Metabolism of AAAs illustrates another emerging function of the gut microbiota, modulation of the neuroendocrine communication between the digestive and nervous systems. The serum concentration of serotonin is significantly reduced in GF mice, but restored when their GI tract is colonized with *Clostridium* species [28]. A similar study on catecholamines found that their levels were similar in GF and conventionally raised (CONV-R) mice; however, the catecholamines in GF mice were mostly in biologically less active conjugated form [29]. Colonizing the GF mice with CONV-R fecal isolates dramatically increased the levels of free catecholamines, suggesting that the gut microbiota could modulate systemic catecholamine levels through deconjugation activity.

Bile acids

Microbial deconjugation also plays an important role in regulating bile acids. These cholesterol-derived metabolites facilitate intestinal absorption of dietary lipids and fat-soluble vitamins, while their rate of synthesis influences systemic cholesterol levels. Synthesis of primary bile acids, cholate (CA) and chenodeoxycholate (CDCA), occurs in the liver. Before entering the intestine, the primary bile acids undergo conjugation with either glycine or taurine, which enhances the bile acids' surfactant function. More than 95% of the bile acids are reabsorbed in the distal ileum and returned to the liver as part of the enterohepatic circulation (Fig. 1). The remainder enters the cecum and colon, where they can be transformed into secondary bile acids. Major secondary bile acids include deoxycholate (DCA) and lithocholate, generated through 7 α -dehydroxylation of CA and CDCA, respectively (Fig. 4a). The enzyme system catalyzing this dehydroxylation step has been found in several *Firmicutes* species, notably *Clostridium scindens* [30]; however, the general distribution of this system in colonic microbiota remains poorly characterized. In contrast, the distribution of bile salt hydrolases (BSHs), which regenerate primary bile acids through deconjugation, has been extensively characterized [31]. Phylogenetic groups encoding BSH include microbiota genera belonging to *Firmicutes* (*Lactobacillus*, *Clostridium*), *Actinobacteria* (*Bifidobacterium*), and *Bacteroidetes* (*Bacteroides*). Together, the deconjugation and dehydroxylation reactions greatly diversify the bile acids found in the intestine [32].

A major component of the host's bile acid signaling system is the farnesoid X receptor (FXR), a transcription factor expressed in both the liver and intestine. Liver bile acid synthesis is under negative feedback regulation by intestinal FXR through a fibroblast growth factor (FGF15)-dependent mechanism. In mice, FXR is antagonized by tauro-conjugated muricholate (T β MCA; MCA is a primary bile acid in mice). The levels of MCA and T β MCA in the distal small intestine are significantly higher in GF compared to CONV-R mice [32]. As a result, FXR-dependent inhibition of bile acid synthesis is impaired in GF mice, consequently increasing cytochrome P450 7A1 (CYP7A1)-catalyzed cholesterol metabolism in the liver [33]. Using intestine specific FXR^{-/-} mice, Li et al. showed that inhibition of FXR signaling correlates with depletion of *Lactobacillus* and *Clostridium* species and with decreases in BSH and 7 α -dehydroxylase activities [34]. Similar correlations were observed when bacterial BSH variants were overexpressed in the intestines of GF or CONV-R mice [35], demonstrating that even a subtle shift in BSH activity impacts the intestinal bile acid profile and host lipid metabolism.

Another role for microbiota metabolism of bile acids is to inhibit colonization by gut pathogens. In its vegetative state, *C. difficile* is susceptible to secondary bile acid (e.g. DCA) toxicity. Employing mouse models, clinical studies, metagenomic analyses, and mathematical modeling, Buffie et al. found that resistance to *C. difficile* infection strongly correlates with presence of *C. scindens*, and showed that *C. scindens* inhibits *C. difficile* growth in a bile salt dependent manner [36].

Choline

In humans, dietary uptake of choline is necessary to maintain a sufficient level for biosynthesis of phospholipids. Metabolomic data indicate that certain species of the gut microbiota carry out anaerobic choline metabolism to produce trimethylamine (TMA), acetate and ethanol (Fig. 4b). Dysbiosis leading to aberrant choline metabolism has been proposed as potential contributing factor in non-alcoholic fatty liver disease, and increased TMA in circulation has been identified as a risk factor for cardiovascular disease and colon cancer. The specific microorganisms responsible for choline metabolism remained largely unidentified until recently, when the choline utilization (*cut*) gene cluster was discovered in sulfate-reducing bacteria. Functional characterization of this gene cluster in human gut bacteria showed that the gene encoding TMA-lyase (*cutC*) was widely distributed across different phyla, but the distribution did not correlate with phylogeny, suggesting that the pathway may have been acquired in some strains via horizontal gene transfer [37]. The study also found that every human gut isolate experimentally confirmed to metabolize choline possesses *cutC*, demonstrating the utility of the gene as a functional marker of choline metabolizing capacity while also underscoring the need to complement genomic and metagenomic analyses with molecular and biochemical characterization.

Methods and Models

Manipulation of gut microbial populations

Due to ethical concerns and practical limitations of manipulating the gut microbiota in human subjects, GF and gnotobiotic mice have featured prominently as experimental model systems. Administration of broad-spectrum antibiotics via drinking water has also been used, but the results can be difficult to reproduce. While gavage feeding of the antibiotics improves reproducibility [38], this can severely stress the animal, presenting difficulties for long-term studies. Another strategy for characterizing the metabolism of specific gut microbes is to conduct *in vitro* cultures, which affords experimental control over substrates and environmental parameters (e.g. pH). Utilizing anaerobic cultures in conjunction with gnotobiotic mice, Goodman et al. showed that the taxonomic groups dominant in human fecal microbiota are represented in its readily cultured members [39]. An intriguing extension of this approach is to introduce culture-expanded isolates into gnotobiotic mice to study the interaction between specific bacterial groups and diet. Additionally, GF mice can be colonized with isolates from individuals with different health status, e.g. obese vs. lean, to study the effects of gut bacteria on the host's metabolic phenotype [40]. Limitations remain, however, with respect to the ability to recapitulate the spatial distribution of microorganisms (site specific clustering [41]) along different parts of the digestive tract. Conventional culture systems grow the cells in a mixed environment, and thus cannot provide this feature. In this regard, the development of staged bioreactor systems [42] or engineered intestinal tissue models supporting stable colonization by bacteria would be immensely useful.

An alternative to isolating specific bacterial subgroups is to characterize the metabolite profile of bodily fluids or intestinal samples, and integrate these profiles with metagenomic data to investigate the microbial origin of the detected metabolites. Untargeted analyses using high-resolution mass spectrometry paired with comparisons using GF or gnotobiotic mice have been especially useful in identifying metabolites whose levels depend on the microbiota and thus reflect bacterial metabolic activity [43]. Due to the high dimensionality of these data sets, multivariate statistical methods are often employed to select discriminatory metabolites that characterize significant differences between samples. However, challenges remain in determining whether a metabolite is the product of host or microbiota metabolism, as many metabolites can be produced in both mammalian and bacterial cells. Moreover, the metabolites present in the intestine could result from co-metabolism involving reactions from multiple organisms [44,45], including the host.

Metabolic models

Analysis of metabolomic data can be facilitated by computational methods that utilize metabolic models. Metabolites identified from an untargeted analysis could be mapped to known pathways via reaction definitions available in databases, which in turn provides a link to enzymes, genes, and genomes. Using pathway analysis, routes could be traced through a metabolic model of the gut microbiota to determine the organism(s) harboring the necessary genes and thus capable of producing the metabolites of interest. Current modeling efforts can be broadly grouped into two approaches. In the first approach, genome-scale metabolic models (GEMs) are built for specific microorganisms, and analyzed using simulation frameworks such as constrained optimization to characterize the organism's metabolic capacities. For example, Heinken et al. utilized a GEM of *B. thetaiotaomicron* in conjunction with flux balance analysis to find that co-metabolism with the bacteria could rescue a potentially lethal loss of enzymatic function in the host [46]. More recently, Shoaie et al. utilized GEMs to simulate metabolic interactions across three representative species of gut bacteria (*Eubacterium rectale*, *Methanobrevibacter smithii*, and *B. thetaiotaomicron*) under varying nutrient settings [47]. In the second approach, the gut microbiota is modeled as a single "super" organism that represents the metabolic capability of an entire microbial community. This approach has the drawback that interactions between particular species cannot be examined detail. However, the community-level models can be constructed in a site-specific manner,

comprising a subset of microorganism residing in the same region of the intestine. Moreover, these models can be directly mapped to metagenomic data to comprehensively analyze the diversity of metabolic functions encoded by the intestinal microbiome [43,48]. Recently, Greenblum et al. assembled a community-level model of human gut microbiota to find significant alterations in the functional organization of the microbiota metabolic network in obesity and IBD [49]. In our recent work, we adopted a similar approach to model the gut microbiota and its host as two intersecting reaction networks, and applied a probabilistic search method to identify pathways of AAA catabolism, while discriminating between microbiota and host reactions [25].

Conclusions

Microbiome research continues to advance at a rapid pace, and significant progress has been achieved towards understanding the metabolic functions performed by the gut microbiota. However, many questions remain regarding the enzymatic pathways and species responsible for the metabolites that mediate host-microbiota interactions. Moreover, it is likely that the metabolites characterized to date represent only a fraction of the bioactive chemicals produced by the microbiota. A recent analysis by the Fischbach laboratory identified more than 3,000 small-molecule biosynthetic gene clusters in bacterial genomes associated with humans [50], highlighting the enormous potential to discover novel bioactive molecules in the metabolome of the gut microbiota.

Several promising directions for future research emerge from the examples discussed above. First, functional characterization of microbiota pathways will benefit from workflows that integrate multiple analytical approaches, including not only omics strategies, but also molecular assays to confirm biochemical function. Second, context-based analyses, e.g. targeting a set of genes that constitute a pathway, will likely yield more robust results than searching for a single gene. This also applies to analysis of metabolomic data, as detection of multiple intermediates of a given pathway, rather than only the end product, will improve confidence that the pathway is active. Third, both data integration and context-based analysis will benefit from metabolic models that comprehensively describe the diversity of pathways supported by a microbial community while also capturing species-specific contributions. Here, the ability to reflect compositional changes in simulations of metabolic outputs would be particularly useful, as this would enable *in silico* predictions on microbiota structure-function relationships leading to experimentally testable hypotheses. Finally, methods and systems will be needed to modulate the activities of specific microbiota pathways *in vivo*, e.g. using genome edited synthetic probiotic organisms, thereby establishing a route to manipulate a metabolic function of interest. These developments as well as others not discussed here will provide new insights into the molecular mechanisms of host-microbiota interactions, while helping to pave the way for rational design of pre-, pro-, and postbiotics beneficial for human health.

Acknowledgements

This work was in part supported by grants from the National Institutes of Health (R21 GM106251) and National Science Foundation (1264502).

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Figure Legend

Figure 1: Representative dietary inputs and metabolic functions of intestinal microbiota. Metabolic functions are indicated in *italics*. Closed and open arrows indicate flow of specific metabolites and dietary residues, respectively. Dotted lines illustrate negative feedback of hepatic bile acid synthesis under the regulation of intestinal farnesoid X receptor (FXR), which is antagonized by conjugated bile acids.

Figure 2: Major pathways of propionate (green arrows) and butyrate (blue arrows) metabolism in gut bacteria. For clarity, only a subset of the intermediates and reactions are shown. Enzyme name abbreviations are shown in bold *italics*. Dotted and solid arrows indicate multiple and single reaction steps, respectively. PDU: propionaldehyde dehydrogenase; MMD: methylmalonyl-CoA decarboxylase; GCD: glutaconyl-CoA decarboxylase; CRO: crotonase; 4HBT: 4-hydroxybutyrate CoA-transferase; KAL: 3-aminobutyryl-CoA ammonia-lyase; BCD: butyryl-CoA dehydrogenase.

Figure 3: (a) Tryptophan (TRP) co-metabolism by host and microbiota. Enzyme name abbreviations are shown in bold *italics*. Blue and red fonts indicate strictly bacterial and host enzymes, respectively. ARAT: aromatic amino acid transferase; IPD: indolepyruvate decarboxylase; TRD: tryptophan decarboxylase; MAO: monoamine oxidase; ALDH: IAAld dehydrogenase; AMI: amidase; TMO: tryptophan 2-monooxygenase. See text for additional definitions of abbreviations. (b) TRP catabolism in gut bacteria produces TrA and IAA, which can translocate across intestinal epithelial cells (IECs) to activate the arylhydrocarbon receptor (AhR) expressed in innate lymphoid cells. This in turn induces secretion of interleukin-22 (IL-22), which triggers an immune response against pathogens, e.g. resulting in production of antimicrobial peptides by Paneth cells.

Figure 4: (a) Key steps in host-microbiota co-metabolism of bile acids. Enzymatic steps are shown in bold *italics*. Blue font indicates bacterial reactions taking place in the intestine. Note that deoxycholate, a secondary bile acid formed by the microbiota, can enter the liver through the enterohepatic circulation and then conjugated with glycine or taurine to a bile salt. See text for definitions of abbreviations. (b) Choline co-metabolism by the host and microbiota. Dietary choline is metabolized in the intestine by bacterial trimethylamine (TMA)-lyase to yield TMA, ethanol, and acetate. Blue font indicates bacterial enzyme. In the liver, TMA is further metabolized into trimethylamine N-oxide (TMAO) by a flavin-containing monooxygenase (FMO). Additional metabolic products of TMA include dimethylamine (DMA); however, the responsible enzyme remains to be elucidated. Choline can also enter the liver, phosphorylated by choline kinase (CKI), and activated to form a substrate for phosphatidylcholine synthesis.

Figure 1

Figure 2

Figure 3

Figure 4