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Chemical communication in the gut: Effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens

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ABSTRACT

Gastrointestinal pathogens must overcome many obstacles in order to successfully colonize a host, not the least of which is the presence of the gut microbiota, the trillions of commensal microorganisms inhabiting mammals' digestive tracts, and their products. It is well established that a healthy gut microbiota provides its host with protection from numerous pathogens, including *Salmonella* species, *Clostridium difficile*, diarrheagenic *Escherichia coli*, and *Vibrio cholerae*. Conversely, pathogenic bacteria have evolved mechanisms to establish an infection and thrive in the face of fierce competition from the microbiota for space and nutrients. Here, we review the evidence that gut microbiota-generated metabolites play a key role in determining the outcome of infection by bacterial pathogens. By consuming and transforming dietary and host-produced metabolites, as well as secreting primary and secondary metabolites of their own, the microbiota define the chemical environment of the gut and often determine specific host responses. Although most gut microbiota-produced metabolites are currently uncharacterized, several well-studied molecules made or modified by the microbiota are known to affect the growth and virulence of pathogens, including short-chain fatty acids, succinate, mucin *O*-glycans, molecular hydrogen, secondary bile acids, and the AI-2 quorum sensing autoinducer. We also discuss challenges and possible approaches to further study of the chemical interplay between microbiota and gastrointestinal pathogens.

1. Introduction

Bacterial pathogens face a difficult task in colonizing the mammalian digestive tract. Not only must they withstand assault from the host immune system and tolerate the presence of toxic chemicals such as hydrochloric acid and bile salts, pathogens must also contend with the resident gastrointestinal microbiota – the community of commensal microbes inhabiting the gut. The microbiota collectively includes all forms of microbial life, including viruses, archaea, and eukaryotes; however, bacteria are the most numerous and also the best studied. Considering that the human digestive tract is estimated to contain up to 10^{14} bacterial cells [66], pathogens face fierce competition for available nutrients. In addition, some gut microbiota species engage in interbacterial warfare, using weapons such as bacteriocins and type VI secretion systems to kill competitors, including pathogens [56,61]. In this review, we focus on a third way that the gut microbiota can affect the success of bacterial pathogens – through its impact on the gut metabolome.

The gut metabolome is the collection of all low-molecular-weight metabolites (~50–1500 Da) found in the gastrointestinal tract [6]. The composition of the gut metabolome is shaped by a complex interplay between the host, microbiota, diet, and xenobiotics such as drugs [6]. There are numerous ways in which the microbiota can affect the gut metabolome, such as by secreting products of bacterial metabolism, producing enzymes that modify host or dietary metabolites, or altering host metabolism through immune signalling [6]. Several recent studies have revealed the dramatic impact of the microbiota on the gut metabolome. When

Abbreviations: EHEC, enterohemorrhagic *Escherichia coli*; SCFA, short-chain fatty acid; T3S(S), type III secretion (system); SPI-1, *Salmonella* pathogenicity island 1; LEE, locus of enterocyte effacement; LCM, low-complexity microbiota; QS, quorum sensing; AI(-2), autoinducer(-2); CAI-1, cholera autoinducer 1; TCP, toxin-coregulated pilus.

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Ref. [50] compared the colonic luminal metabolome of germ-free (microbiota-lacking) and conventionally raised (microbiota-possessing) mice, they found that a majority (123/179) of detected metabolites were altered in abundance by the presence of the microbiota. Another study that compared the metabolomes of germ-free, conventional, and "humanized" mice (germ-free mice colonized with human microbiota) found significant differences even between conventional and humanized mice, indicating that some metabolic processes differ even between the highly related human and mouse gut microbiota [47]. *In silico* techniques have also been used to explore the metabolic potential of the gastrointestinal microbiota. Using available genome sequences of both the mammalian host and cultured gut microbiota species, [70] identified a total of 3449 metabolic reactions predicted to occur in the gut, of which 1267 were uniquely performed by microbiota. A similar *in silico* approach has been used to identify biosynthetic gene clusters present in human microbiota reference genome sequences [20]. The authors found an average of 599 biosynthetic gene clusters in each gut metagenome dataset from the Human Microbiome Project, 519 of which appear to be relatively conserved between individuals (found in more than 50% of samples). In the vast majority of cases, the molecule produced by the enzymes encoded in the biosynthetic gene cluster is currently uncharacterized. Together, these studies show that the microbiota shapes the chemical environment of the gut, producing a multitude of characterized and uncharacterized metabolites whose effects on the host and other microbes are only beginning to be elucidated.

2. Evidence for effects of gut microbiota metabolites on bacterial pathogens

It has long been recognized that an intact gut microbiota protects the host against infection by gastrointestinal pathogens, a phenomenon known as colonization resistance [9,35,69,81]. The microbiota can mediate these protective effects both through direct microbe–microbe interactions and through indirect effects on the host (such as modulating immune functions or altering the expression of host receptors for bacterial toxins). In this review, we focus on the direct effects of microbiota metabolites on bacterial pathogens; indirect effects of the microbiota on the immune system have recently been reviewed elsewhere [1,8].

One line of evidence that microbiota metabolites affect the outcome of bacterial infection relates to the increased susceptibility of humans and animals treated with antibiotics to a variety of infectious agents [37]. For example, when *Salmonella enterica* serovar Typhimurium is administered orally to mice, it invades through the gut epithelium to cause a systemic, typhoid-like infection with minimal intestinal colonization. However, when mice are pretreated with streptomycin, they become susceptible to gastroenteritis (intestinal inflammation and diarrhea), with high levels of *S.* Typhimurium colonization and inflammation in the cecum [5,22,64]. Although the mechanisms underlying this increased susceptibility to gastroenteritis are not fully understood, it is known that streptomycin treatment causes a drastic change in the gut metabolome. Untargeted mass spectrometry experiments found that a single dose of streptomycin caused changes in abundance of 87.8% of the more than 2000 metabolite features detected in mouse feces, with major changes in the levels of primary bile acid, steroid hormone, and eicosanoid hormone metabolites [3]. Pre-treatment with antibiotics including cefoperazone and clindamycin also facilitates *Clostridium difficile* colonization of the mouse gut [34,57]. Two recent studies showed major shifts in the abundance of bile acids, sugar alcohols, and short-chain fatty acids in the ceca and feces of mice treated with these antibiotics [34,73]. Further *in vitro* studies demonstrated that these metabolites affect the growth of *C. difficile*. For example, the secondary bile acid deoxycholate, which was found to be less abundant in antibiotic-treated mice, inhibited *C. difficile* spore germination, while the primary bile acid taurocholate, which increased in abundance after antibiotic treatment, promoted spore germination [73]. Furthermore, *C. difficile* could use some of the sugar alcohols whose abundance was dramatically increased by antibiotic treatment, such as mannitol and sorbitol, as a carbon source for growth *in vitro* [73]. Although the specific microbiota species driving changes in metabolite abundance were not identified in these studies, they provide strong evidence of a link between the microbiota, gut metabolism, and the outcome of pathogenesis.

Further evidence for effects of gut microbiota metabolites on bacterial pathogens arises from studies using individual cultured microbiota isolates. For example, [65] screened human fecal samples for their ability to inhibit the growth of *Vibrio cholerae in vitro*. By screening microbiota isolates cultured from one inhibitory fecal sample, the authors identified a *Peptostreptococcus sp.* and a *Lactobacillus sp.* that both secreted unidentified metabolites that inhibited *V. cholerae* growth *in vitro* and *in vivo* in gnotobiotic mice. Two other studies focused on microbiota metabolites that inhibit virulence gene expression rather than pathogen growth. [63] found that spent culture medium from human fecal microbiota repressed the expression of the enterohemorrhagic *Escherichia coli* (EHEC) *stx2* gene, which encodes the Shiga toxin that causes haemolytic uremic syndrome and kidney failure in a fraction of EHEC-infected humans [17]. After screening individual culture supernatants from a collection of microbiota isolates, the authors determined that *Bacteroides thetaiotaomicron* secreted a small molecule (<3 kDa) that strongly repressed *stx2* expression. More recently, [4] used transcriptomics to identify *S.* Typhimurium genes activated or repressed by metabolites present in an organic extract of human feces. Among the dozens of genes that were affected by the presence of these metabolites, the authors found upregulation of numerous motility and chemotaxis genes, while many of the downregulated genes were involved in invasion of host cells. This repression of invasion genes was a common trait among fecal extracts from different hosts, since the repression could be reproduced with feces from nine out of ten human donors and from two different mouse strains. The inhibitory effect was also observed with culture supernatants of individual gut microbiota isolates, particularly *Clostridium citroniae* and other representatives of the Lachnospiraceae, demonstrating that it is microbiota-generated metabolites and not host factors that are responsible for repressing *Salmonella* invasion gene expression. Even though none of these three studies identified the specific metabolite responsible for growth inhibition or virulence gene repression, together they establish that microbiota-secreted metabolites have appreciable effects on the behaviour of a variety of pathogens.

In the following two sections, we describe cases where the individual metabolites that affect bacterial pathogens have been identified – the first covers metabolites promoting infection, while the other covers those inhibiting infection. We recognize that the effects of these metabolites can vary according to their concentration and the pathogen examined; therefore, we have categorized the metabolites according to their best-studied effects.

3. Microbiota metabolites promoting bacterial infections

3.1. Short-chain fatty acids

Short-chain fatty acids (SCFAs) are among the most abundant products of bacterial fermentation in the gut, reaching concentrations of 50–150 mM in the human colon [41]. The principal SCFAs produced by gut microbiota are acetate, propionate, and butyrate,