



Bacterial Chat: Intestinal Metabolites and Signals in Host-Microbiota-Pathogen Interactions

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ABSTRACT Intestinal bacteria employ microbial metabolites from the microbiota and chemical signaling during cell-to-cell communication to regulate several cellular functions. Pathogenic bacteria are extremely efficient in orchestrating their response to these signals through complex signaling transduction systems. Precise coordination and interpretation of these multiple chemical cues is important within the gastrointestinal (GI) tract. Enteric foodborne pathogens, such as enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella enterica* serovar Typhimurium, or the surrogate murine infection model for EHEC, *Citrobacter rodentium*, are all examples of microorganisms that modulate the expression of their virulence repertoire in response to signals from the microbiota or the host, such as autoinducer-3 (AI-3), epinephrine (Epi), and norepinephrine (NE). The QseBC and QseEF two-component systems, shared by these pathogens, are involved in sensing these signals. We review how these signaling systems sense and relay these signals to drive bacterial gene expression; specifically, to modulate virulence. We also review how bacteria chat via chemical signals integrated with metabolite recognition and utilization to promote successful associations among enteric pathogens, the microbiota, and the host.

KEYWORDS chemical signaling, *Enterobacteriaceae*, *Escherichia*, *Salmonella*, intestinal metabolites

COMMENSALS AND PATHOGENS IN THE GUT

The large and diverse bacterial community in the human gut also has important functions in the physiology of the intestine. The intestinal mucosa forms a physical barrier that keeps the microbiota on the luminal side. The mucus layer is composed of mucin, glycoproteins, trefoil peptides, and surfactant phospholipids (Fig. 1). Altogether, they constitute a nutrient-rich mucus layer, which has an important role as a protective barrier against microorganisms (1). The biogeography of the gastrointestinal (GI) tract is diverse in its composition and density and its distinct chemical and physical features (2). The density and composition of bacterial communities change according to their location in the gut. Throughout the GI tract, there is significant variation in the physicochemical conditions and substrate availability that impact bacterial growth, differentially promoting or hampering the colonization of certain niches by various species. In the proximal colon, the high concentration of sugar substrates in the mucus allows the expansion of the saccharolytic members of the microbiota (Table 1). Inversely, the lower availability of sugar substrates in the distal colon triggers proteolysis, which decreases the bacterial growth rate and the diversity of the microbiota (1, 3). The resident microbiota, together with all chemical and physical features of the intestine, contribute to shape the metabolic landscape within the gut, producing a multitude of characterized and as-yet-unknown intestinal metabolites. Moreover, the microbial composition of the human GI tract varies with age, diet, host genetics, and external insults like antibiotic treatments (4, 5).

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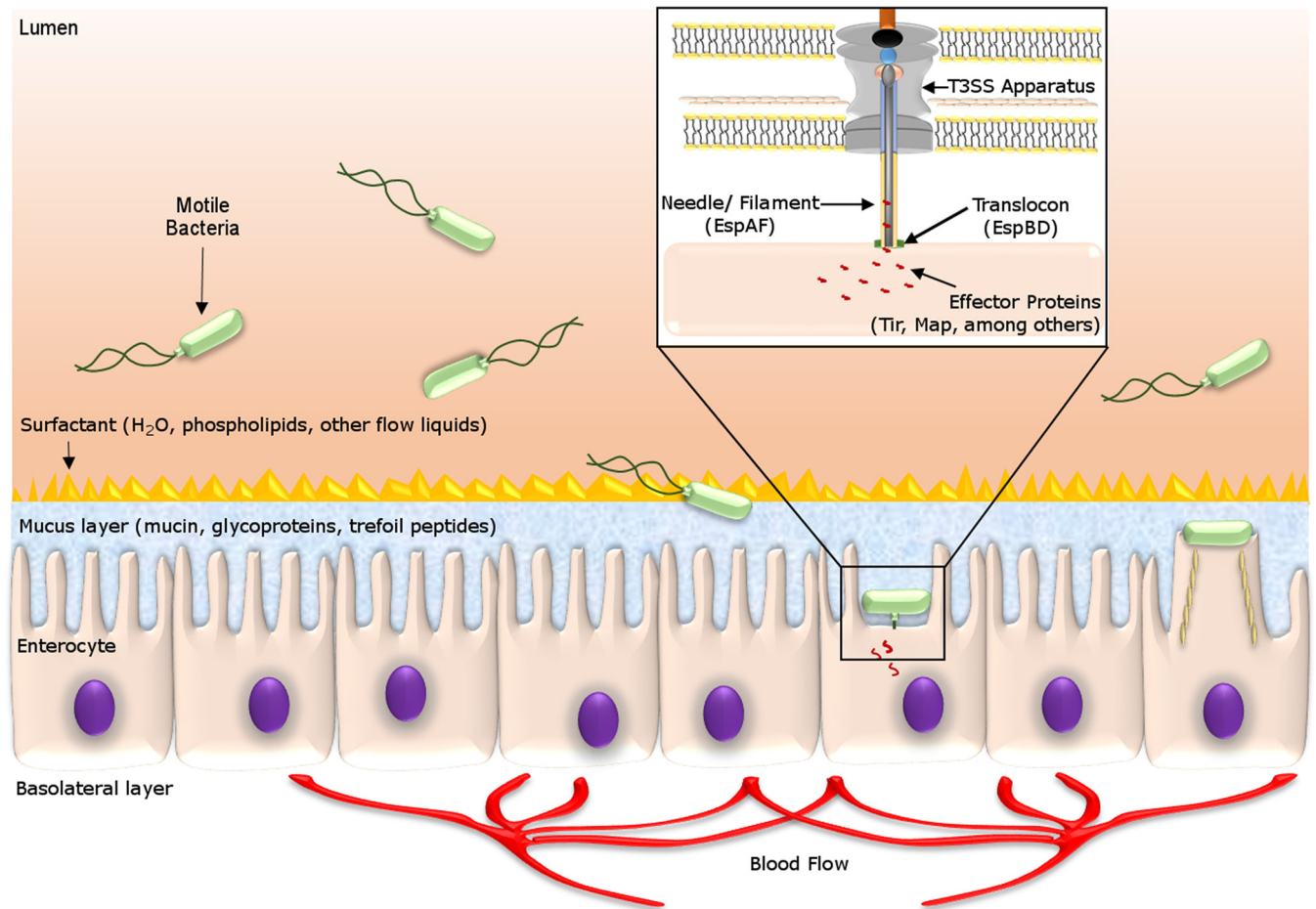


FIG 1 The epithelial layers and compounds in the human small intestine comprise a rich environment for bacterial species and also display an important mucosal barrier that separates enterocytes from the luminal environment. After passing the mucosal layer and finding the enterocytes, EHEC expresses a T3SS, which forms a structure similar to a needle that allows the bacteria to inject secreted proteins into host cells. These proteins orchestrate changes in the actin and myosin filaments that promote the formation of the pedestal that characterizes the A/E lesion (14, 39).

The intestinal environment hosts a diverse microbial community that modifies and shapes the composition of the metabolites and chemical signals within the gut. Pathogenic bacteria employ multiple systems to sense and respond to these cues to interact with the microbiota and the host. The ratio between bacteria and host cells in the human body is still unresolved; previous studies have estimated a 10:1 ratio (6–8), although recently it was calculated to be closer to a 1:1 ratio (9). However, this does not change the important role of microbiota-host interactions (10) and may reflect the microbiota’s fluctuation in this association. The intestinal microbiota is thought to be an

TABLE 1 The different physicochemical conditions and substrate availabilities for bacterial growth along the gut to favor the colonization of beneficial bacteria

Section of colon	Characteristic ^a
Proximal	High concn of sugars and substrates Saccharolysis pH of approximately 5.0 to 6.0 High bacterial growth and diverse microbiota
Distal	Low availability of substrate Proteolysis Neutral pH Low bacterial growth and microbiota diversity

^aData are from references 1 to 3.

essential contributor to human health. However, despite the protection offered by different commensal bacteria, pathogens are capable of invading and exploiting this balanced system. These pathogens compete for nutrients and hijack general metabolites from the host or the resident microbiota, employing them as signals (6, 7).

The balance between commensal and potentially pathogenic bacteria is a central element of human health. The microbiota benefits the host by enabling fermentation of nondigestible diet components, such as complex sugars and lipid molecules. This metabolic breakdown leads to vitamin K production, absorption of important ions, and changes in basic cell functions, such as controlling the proliferation and differentiation of epithelial cells. The microbiota also impacts the functioning of the immune system (1, 4, 5).

Enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella enterica* serovar Typhimurium are two major foodborne pathogens that cause gastroenteritis outbreaks worldwide (11–14). EHEC is an important human pathogen that colonizes the large intestine but is a commensal in other hosts, such as cattle, its main reservoir (15). Intestinal disease caused by EHEC cannot be replicated in mice with the same clinical aspects as observed in human infections. Thus, an important surrogate murine infection model is *Citrobacter rodentium*, a natural murine pathogen that shares many virulence traits with EHEC (16). *S. Typhimurium* also causes gastroenteritis, but unlike EHEC, it may progress to systemic infection (13, 17). Recent studies investigating the relationship between these pathogens and covering the resident microbiota have started to elucidate how these pathogens outmaneuver the host defenses.

FROM FOOD SOURCES TO MICROBIAL METABOLISM

The composition of the intestinal microbiota is impacted by diet, lifestyle, and host genetics. Diet influences nutrient availability in the gut and changes the composition of the intestinal microbiota. Pathogenic bacteria generally compete directly against commensals for nutrients and colonization sites within the intestine (18, 19).

Fermenter bacteria present in the proximal colon, such as members of the *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* phyla, break down more complex dietary carbohydrates. These organisms produce short-chain fatty acids (SCFAs), particularly acetate, propionate, and butyrate. These metabolites are important energy sources that aid host cell differentiation and nutrient absorption by the colonic epithelium (3, 18, 20).

Mice fed with acetylated starch have increased bacterial acetate levels in their feces, leading to protection against an initial EHEC colonization (21). Moreover, EHEC-infected mice coinfecting with *Bifidobacterium longum*, which has a subset of carbohydrate transporters, can produce enough acetate via bacterial sugar metabolism to promote host defenses against enteric pathogens (21). Details are discussed further in Metabolites Influencing EHEC Pathogenesis below.

THE CHALLENGE OF GI TRACT COLONIZATION

Enteric bacteria face many challenges in colonizing the GI tract. These organisms must survive the host immune defenses and tolerate the presence of toxic chemicals, including hydrochloric acid and bile salts. In addition to host immune defenses, specific cues, dependent on the intestinal niche location and infection timing, play a role in gene expression that allows bacteria to thrive during this competition. Bacterial acid resistance systems allow them to survive several hours at low pHs, ranging between 2.0 and 6.0, during the passage through the human stomach (22). High tolerance to bile is another critical characteristic for GI bacteria, as the bile in the small intestine is detrimental to bacterial survival (23). Bacteria employ multiple systems to be able to survive, including efflux pump systems that secrete toxic compounds; in parallel, these organisms may also trigger changes in bacterial membrane permeability to avoid the excess of some ions. Another stress that bacteria in the GI tract must resist is cationic antimicrobial peptides found in the gut. Gram-negative bacteria may chemically change their lipid A chains, inducing distinct lipopolysaccharide layer modifications to

fortify the bacterial defenses as a shield to resist antimicrobial peptides (24). Surviving differences in oxygen levels is also a key ability for bacteria that inhabit the GI tract. The GI tract features variable oxygen levels, from a relatively anaerobic lumen to a more oxygenated area adjacent to the mucosal surface that is generated by diffusion from the capillary network of the microvilli. The ability of enteric bacteria to switch from aerobiosis to anaerobiosis or microaerobiosis is advantageous (22, 23). Bacteria employ multiple strategies to colonize the inhospitable GI tract. By sensing different metabolites, bacteria successfully colonize the gut; unfortunately, among them, pathogenic bacteria also have metabolite-sensing systems to compete against the microbiota.

BACTERIAL METABOLITE SENSING

Bacteria need to adapt their metabolism quickly to survive hostile environments. Metabolite-sensing mechanisms are found both in commensal and pathogenic bacteria. Commensal *E. coli* colonizes the human GI tract within a few hours after birth, initiating a mutually beneficial relationship (14). Pathogenic *E. coli* employs complex virulence mechanisms that are activated by chemical signaling and nutrients, both essential for intestinal colonization and evasion of host defenses (19, 25).

EHEC and *S. Typhimurium* employ cell-to-cell communication to regulate their virulence programs and cause gastroenteritis (12–14). Similarly, *Citrobacter rodentium* uses these cell-cell communication systems to trigger intestinal colitis in mice (16). These enteric pathogens have in common an intricate signaling system employing cues from both the host and the gut microbiota. The chemical and nutrient signaling in these bacteria is mediated by two-component systems (TCS), such as QseBC and QseEF, that sense these differences and initiate a complex signaling cascade that drives the virulence program in these pathogens (Fig. 2) (15, 19). The bacterial enteric community is a complex system and suffers constant exposure to threats, such as potential pathogens.

Enterics maintain a successful homeostatic community, but the resident microbiota may be manipulated by bacterial pathogens, such as EHEC. EHEC pathogenesis is characterized by a low infectious dose, and therefore, only a few CFU are enough to colonize the host. EHEC scavenge metabolites from different microorganisms, such as *Bacteriodes thetaiotaomicron*, a highly prevalent saccharolytic bacterium in the intestine. *B. thetaiotaomicron* can break down plant starches and other complex carbohydrates to simpler sugar molecules that can be utilized by other members of the microbiota (26, 27). For example, fucose released from the mucus by *B. thetaiotaomicron* can be used by pathogens like EHEC to trigger their infectious processes (28).

BACTERIAL PATHOGENESIS CONTROLLED BY INTESTINAL CHEMICAL SENSING

E. coli O157:H7 is the most common and serious EHEC serotype that causes human illness. Its lethality is caused by the presence of the potent Shiga toxin, carried on a lambdoid prophage. This toxin can cause hemolytic uremic syndrome (HUS), a life-threatening condition (14, 29, 30). EHEC harbors a pathogenicity island named the locus of enterocyte effacement (LEE), which encodes a microscopic “needle,” a type III secretion system (T3SS), that injects many different effector proteins into the host cell (Fig. 1) (19, 28, 31–35). In addition to the T3SS, the LEE also contains genes that encode an adhesin (intimin) (36) and its own receptor, the translocated intimin receptor (Tir) (37, 38), which is translocated into the epithelial cell (33–35). Together, these effectors lead to extensive host cytoskeletal rearrangements and intestinal infection (14, 39).

Animal models of EHEC infection are limited, and they do not mirror all of the disease facets, such as the low infectious dose that is enough to trigger the diarrheagenic disease (12). Different EHEC infection models have been tested in very distinct animals, such as ferrets, greyhound dogs, monkeys, *Caenorhabditis elegans*, rats, baboons, and chickens (40–46), but these models reproduce only a few aspects of the disease. The models most used to mimic EHEC pathogenesis are pigs, rabbits, and mice (47). Briefly, piglets are used in EHEC infections. However, these infections do not result in attaching and effacing (A/E) lesions; gnotobiotic piglets show a watery but not

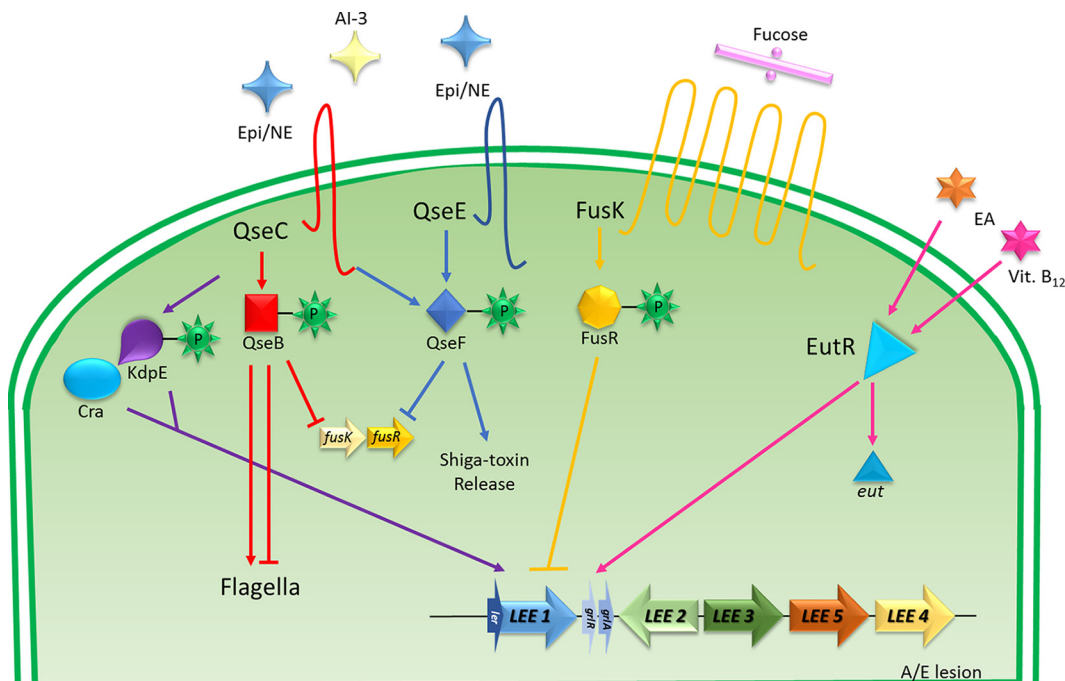


FIG 2 Chemical signaling by Epi/NE, Al-3, fucose, and ethanolamine (EA) in EHEC interacts with transmembrane sensor kinase receptors and activates a TCS via response regulators, which may activate or repress the signal transduction. The QseC sensor kinase has a central role in the activation of the QseB response regulator by Epi, NE, or Al-3; QseB regulates the expression of flagella and represses the expression of *fusK/R*. QseC can also phosphorylate KdpE, which together with Cra, activates the pathway responsible for LEE activation. QseC also may activate the response regulator QseF, which stimulates the production of Shiga toxin, but it is able to repress the expression of *fusK/R*. The cognate sensor FusK is activated by fucose and phosphorylates FusR to inhibit LEE expression (28, 97, 104, 128). The regulation of the *eut* operon is also implicated: *eut* encodes the EutR transcription factor, which recognizes the presence of ethanolamine (EA) and vitamin B₁₂ to modulate virulence gene expression. EutR can bind in the LEE in the absence of EA or B₁₂, but transcription occurs only in the presence of both. In the presence of EA and B₁₂, *eut* expression promotes EA metabolism, providing an advantage in the competition for nutrients, and also promotes host colonization (129).

bloody diarrhea (48, 49), while conventional animals, such as suckling piglets, exhibit more severe neurological diseases rather than renal toxicity effects from Shiga toxin (50). Rabbits are also employed in different models, such as 3-day-old infant (51), suckling, up to 10-week-old young (52–54), or adult (55) rabbits. Rabbits are susceptible to oral administration of EHEC (52), but they do not develop HUS, since rabbits lack the renal receptor globotriacylceramide (Gb3) for Shiga toxin (56). The 3-day-old infant New Zealand White rabbit infection model is one of the most reproducible animal models for EHEC, with the pathogen reaching consistent levels of colonization in the large intestine, forming A/E lesions, causing diarrhea, and persisting in the intestine, causing a delay in the animals’ growth (51, 57, 58). Conversely, mice have Gb3 receptors, and Shiga toxin causes acute renal damage and death in mice (59, 60). However, mice are naturally resistant to colonization by EHEC, and thus, murine models need a depleted microbiota to allow EHEC colonization (47). Many different mouse models are used to address this issue (60), such as pretreatment with antibiotics (61, 62), germfree conditions (63–65), or diet-induced changes (66), all of which modify the microbiota environment to promote intestinal disease. Despite recent advances in the field, EHEC animal models do not reproduce the full clinical illness seen during human infection (47). Altogether, *C. rodentium* infection in mice appears to be the only natural murine model to study some aspects that EHEC and *C. rodentium* infection have in common (47). *C. rodentium* also harbors the LEE pathogenicity island and forms A/E lesions on the murine gut (67–69). Most of the EHEC virulence genes have been described previously by studying the function of the *C. rodentium* orthologs during murine infection (67–69).

Salmonella is also an important foodborne pathogen. The *Salmonella enterica* sero-

var Typhimurium murine infection models have been extensively applied to study *Salmonella* gastroenteritis (13). The gastroenteritis caused by *S. Typhimurium* requires two steps that are mediated by two distinct T3SSs (70) encoded by two *Salmonella* pathogenicity islands (SPIs), SPI1 and SPI2. The SPI1-encoded T3SS is active during the initial contact with host epithelial cells and translocates bacterial proteins across the host membrane, and the SPI2-encoded T3SS is expressed within the phagosome and translocates effectors across the vacuolar membrane. The SPI1 system is required for invasion of nonphagocytic cells, induction of intestinal inflammatory responses, intestinal colonization, and diarrhea (71, 72). The SPI2-encoded T3SS has an important role in bacterial survival in macrophages and the establishment of systemic disease (73). The *S. Typhimurium* murine infection model has been extensively employed in the study of many virulence traits (71–73).

Intestinal chemical sensing plays an important role in *S. Typhimurium* colonization of the gut in murine models pretreated with antibiotics (74). The changes in the composition of the microbiota promote pathogen expansion mediated by the oxidation of carbohydrates (74). Probiotic bacteria present in the intestinal resident microbiota are key elements to this complex bacterial ecosystem. Members of the *Bacteroidetes* and *Firmicutes* phyla are prevalent anaerobic bacteria in healthy individuals. Both metabolize nondigestible proteins and complex carbohydrates, such as gluten and fiber or mucus saccharides, respectively (75). In the absence of other sources, bacteria rely on fermentation of these carbohydrates and proteins to obtain the carbon and nitrogen that are essential to the biosynthesis of bacterial proteins, carbohydrates, lipids, and nucleotides. Although reactive oxygen and nitrogen species are generated by the inflammatory host response during inflammation induced by *S. Typhimurium*, they oxidize luminal compounds (trimethylamine and thiosulfate), forming exogenous electron acceptors (trimethylamine *N*-oxide and tetrathionate) that can be used by *S. Typhimurium* (76). The presence of these acceptors enables facultative anaerobic bacteria, such as *S. Typhimurium*, to utilize microbiota-derived products to generate energy, balance redox reactions, and acquire carbon for the biosynthesis of other products essential to bacteria during infection (75).

The large majority of human pathogens require iron as a nutrient to support growth and colonization. Iron is essential to all living cells, although a tight concentration control is essential to avoid its toxicity, due to the redox potential; invading bacteria, such as *S. Typhimurium*, have evolved many systems to ensure the uptake of iron that is available intracellularly in the host (77). This important host-pathogen interface is linked to pathogen proliferation, virulence, and persistence (78, 79). *S. Typhimurium*'s ability to grow in the host regardless of the iron level is mediated by Feo proteins, which take up unbound ferrous iron (Fe^{2+}) (77, 80). The proliferation of invasive bacterial pathogens is limited by the bioavailability of iron. The innate immune system is capable of limiting iron availability in the presence of invading microbes, also known as nutritional immunity; however, pathogens possess mechanisms to circumvent this and cause disease (79). During inflammation, iron becomes even more limited, and therefore, *Salmonella* relies on siderophores to bind to ferric iron (Fe^{3+}), sequestering Fe^{3+} from host proteins. Enterobactin is the most common siderophore among the *Enterobacteriaceae*. Salmochelin is another siderophore present in *Salmonella*, a C-glucosylated derivative of enterobactin (81), but different than enterobactin, it cannot bind to the antimicrobial lipocalin-2, which prevents bacterial iron acquisition; thus, salmochelin confers an important advantage to *S. Typhimurium* versus microbiota members during intestinal colonization (82). However, the probiotic *E. coli* strain Nissle is also able to evade iron sequestration by lipocalin-2. *S. Typhimurium* and *E. coli* Nissle compete for iron sources during inflammation (83). In this case, iron uptake and evasion of lipocalin-2 are beneficial to the host, since the competition favors colonization by the probiotic *E. coli* Nissle during *S. Typhimurium* inflammation (83). The nutrient-limiting condition and different bacterial survival strategies are very important players to understand for insight into how pathogens interact with indigenous microbiota and the surrounding metabolites and, specifically, their strategies to explore distinct niches.

METABOLITES INFLUENCING EHEC PATHOGENESIS

Various metabolites and compounds may influence the course of EHEC infection. Recent experiments with EHEC-infected mice suggest that food choice has a significant impact on Shiga toxin expression (66). EHEC-infected mice fed a low-fiber diet exhibited higher weight loss and a lower Shiga toxin-dependent survival rate. These data suggest that a high-fiber diet renders these animals more resilient in the face of Shiga toxin exposure. A high-fiber diet led to a reduction in the commensal *E. coli* population compared to those of other species, which benefits EHEC colonization during gut competition. However, the findings for different levels of dietary fiber are contradictory. Regarding EHEC colonization and Shiga toxin production, this murine model mostly reflects Shiga toxin effects and not the GI phase of infection, because EHEC does not promote A/E lesion formation in the mouse intestine (66). Distinct metabolites are very important to pathogens, while these organisms must scavenge and exploit all signals to quickly adapt themselves during colonization.

The SCFAs have an important role during EHEC pathogenesis, because they impact EHEC gene regulation. Butyrate works as a signal in EHEC by enhancing the expression of the T3SS and flagellar genes. Butyrate activates the flagellar master regulator, *flhDC*, through the leucine-responsive regulatory protein (Lrp), a regulator of virulence genes. Moreover, butyrate, acetate, and propionate also activate downstream genes independently of *flhDC* activation (84, 85).

Other metabolites repress signals and limit EHEC colonization in hostile environments (86). Biotin, present at high levels in the small intestine, reduces the presence of EHEC in this GI compartment. Conversely, the lower levels of biotin found in the large intestine favor EHEC colonization (86). A novel system was recently described to sense D-serine levels, acting to repress the injection of effector proteins into the host cell via the T3SS. This system functions in a concentration-dependent manner via the DIsT/YhaJ D-serine sensor system. D-Serine can either act as a carbon source or modulate a set of stress-dependent genes in *E. coli* strains of different pathotypes and, consequently, modulate the gene expression of unique virulence factors (87).

Other bacterial interactions may be very important during EHEC pathogenesis. Recently, the probiotic bacterium *Bifidobacterium longum* was reported to change the EHEC outcome in the gut through the production of acetate, preventing translocation of the Shiga toxin through the intestinal epithelium and ultimately avoiding HUS. This protection is attributed to a higher acetate concentration accompanied by increased consumption of carbohydrates by this probiotic *B. longum* gut member. *In vitro*, acetate protects the permeability of epithelial cell monolayers, which directly affects EHEC infection, but the complete mechanism is still not fully elucidated (88). The composition of the intestinal microbiota and different metabolites seem to directly affect the GI trespassers (21), which may be an explanation for the differential outcomes of GI tract infections in distinct individuals.

CHEMICAL SIGNALING IN BACTERIA

Bacteria employ different mechanisms to explore the GI tract and sense distinct cues. This chemical signaling among bacteria regulates different aspects of their transcriptomes and is usually referred to as quorum sensing (QS). QS was first described in the environmental *Vibrio fischeri*, a Gram-negative marine bacterium. When *V. fischeri* bacteria are at high cell density in a squid or fish light organ, the bacterial QS system activates their bioluminescence genes within the light organ. This is achieved through diffusible chemicals called autoinducers (AIs). AIs act as hormonelike molecules (89).

THE AI-3-EPINEPHRINE/NOREPINEPHRINE INTERKINGDOM SIGNALING SYSTEM

Among these QS signaling systems, the bacterial AI-3 molecule has been shown to be present in different bacterial species (90) and to have a key role in interkingdom signaling. AI-3 is produced by the human GI microbiota and cross signals with the host hormones epinephrine (Epi) and norepinephrine (NE) (25). The AI-3-Epi/NE system has been implicated in controlling the pathogenesis of EHEC (Fig. 2) (25, 91). This system

has also been shown to promote virulence gene expression in *S. Typhimurium* (57, 92, 93) and *Citrobacter rodentium* (94), among other species (95). Bacteria sense these interkingdom chemical signals through histidine sensor kinases anchored in the bacterial inner membrane. The QseC and QseE receptors are two kinases that have been described to sense these cues (28, 96, 97). QseC and QseE phosphorylate their cognate response regulators (RRs) in the cytoplasm, as well as noncognate RRs, to modulate the expression of the virulence genes in EHEC (Fig. 2) (62, 67).

The stress hormones Epi and NE are derived from tyrosine-containing catechol and amine groups (98). These catecholamines are neurotransmitters of the central and peripheral nervous system that are responsible for regulating many body functions, including the fight-or-flight response, cognitive activity, and mood, as well as functions of the liver, lung, and adipose tissue and the cardiovascular system, and they also affect intestinal peristaltic movements (99, 100). Mammalian cells recognize these neurotransmitters through adrenergic receptors, a subset of the family of G protein-coupled receptors (GPCRs). In contrast, bacterial cells do not encode GPCR receptors in their genomes but have the membrane-bound histidine sensor kinase receptors that are able to sense these hormones (96, 101).

THE QseBC AND QseEF TCSs

The QseBC two-component system (TCS) is found in *Proteobacteria*, including animal and plant pathogens (57, 102, 103). The QseC histidine sensor kinase increases its autophosphorylation in the presence of AI-3–Epi/NE molecules and relays this information to its cognate RR QseB and two noncognate RRs, QseF and KdpE (Fig. 2) (96, 104). The QseBC TCS is involved in chemical signaling that regulates flagella and motility in EHEC (11). QseBC regulates the expression of *flhDC*, which encode the master flagellar regulator. QseB directly activates *flhDC* expression and binds directly to the low- and high-affinity binding sites of the *flhDC* promoter. QseBC initiates flagellar class 1 gene expression, dependent on the presence of *flhDC* FlIA (sigma 28) (101).

TCSs enable bacteria to sense their specific signals but may also trigger their response to different cues; therefore, TCSs can cross-communicate with other TCSs, depending on the specificity of the signal sensor kinases that together form a broader and more complex regulatory system (105). QseC activates its cognate RR QseB, and it also phosphorylates the RRs KdpE and QseF (Fig. 2) (67). The KdpDE system is regulated by the potassium concentration, while the RR KdpE is phosphorylated by the histidine kinase KdpD (106). QseC phosphorylates both KdpE and QseF, activating LEE and Shiga toxin expression (104).

The QseE sensor kinase senses Epi/NE, sulfate, and phosphate (62). QseE only phosphorylates its cognate RR, QseF (97, 107). QseF affects the expression of Shiga toxin (67) and the small RNA GlmY (108, 109), which works in concert with GlmZ to posttranscriptionally regulate the expression of LEE and EspFu (an effector necessary for A/E lesion formation) in EHEC (108, 109).

EHEC and *C. rodentium* share the QseBC and QseEF systems that sense Epi and NE and which regulate their virulence genes similarly (94). Both adrenergic hormones activate virulence in *C. rodentium* via the QseC and QseE sensors. *qseC*, *qseE*, and *qseEC* mutants are attenuated during murine infections (94). QseC and QseE also sense Epi and NE in the mouse intestine, as shown in infection studies with dopamine-hydroxylase knockout (*Dbh*^{-/-}) mice, which are unable to produce Epi and NE (94).

QseC and QseE also regulate the virulence expression and pathogenesis of *S. Typhimurium* in swine and mice (57, 92, 93). QseC regulates the expression of flagellum and motility genes and *sifA* (93). *SifA* is necessary for *S. Typhimurium*'s survival inside macrophages (110). A Δ *qseC* mutant has decreased colonization in swine (92) and is attenuated for systemic infection in mice (93). The *qseE* mutant has reduced invasion of epithelial cells and reduced intramacrophage survival. Additionally, the *qseEC* double mutant is slightly attenuated only in epithelial cell invasion, which mirrors the phenotype of the Δ *qseC* mutant, and it has an intermediate intramacrophage replication defect in comparison to the intramacrophage replication of the *qseE* single mutant. The

QseC sensor kinase participates actively in *S. Typhimurium*'s pathogenesis via the expression of virulence genes and phenotypically during intramacrophage replication as observed *in vivo* and *in vitro* infections.

The expression of the SPI1 T3SS effector genes, such as *sipA* and *sopB*, which occurs during the invasion of epithelial cells, is activated by epinephrine via QseE. The expression of these genes is also decreased in the $\Delta qseEC$ mutant, albeit to a lesser extent than for those described above, congruent with its attenuated-invasion phenotype. The expression of SPI2 T3SS genes, such as *sifA*, is important during intramacrophage replication, which is also decreased in the *qseE* and the *qseEC* mutants. During systemic murine infections, the *qseE* mutant is highly attenuated, while the double mutant has an intermediate phenotype (111). The transcriptional expression levels of both the SPI1 and SPI2 genes during mouse infections show the importance of the QseEF TCS system during *S. Typhimurium* pathogenesis.

In transcriptomic analyses of the QseC regulon, QseC regulates the expression of the virulence stress-related periplasmic protein (VisP). VisP binds to peptidoglycan and interacts with the LpxO-lipid A-modifying enzyme, inhibiting lipid A hydroxylation. VisP promotes LpxO inhibition and, thus, reduction of lipid A hydroxylation, which is important for intravacuolar survival upon stresses like acidification and exposure to hydrogen peroxide and heavy metals like cadmium chloride (112). More studies are necessary for full elucidation of the role of VisP during systemic infection. Nevertheless, both VisP and LpxO also have independent functions during *S. Typhimurium* colitis. VisP integrates chemical signaling, stress responses, and lipopolysaccharide (LPS) modifications during *S. Typhimurium* pathogenesis (112). The QseC and QseE adrenergic sensors modulate several aspects of EHEC, *S. Typhimurium*, and *C. rodentium* pathogenesis. Some arms of these signaling systems converge among these pathogens, while others diverge.

SUGAR REGULATION OF VIRULENCE

Sugars are the primary carbon sources for bacterial cells, and they also play an important role in bacterial pathogenesis (28, 113). Both pathogenic and commensal bacteria compete for carbon sources like glucuronate, mannose, fucose, and ribose to colonize and proliferate in the gut. EHEC uses breakdown products from commensal bacteria, such as mucin-derived sugars, to gain a competitive advantage during intestinal colonization (114). Sugar availability influences the microbiota composition and the expansion of bacterial pathogens (113). The expression of the LEE in EHEC is inhibited during glycolytic metabolism (115). Conversely, EHEC growth within a gluconeogenic environment activates the expression of the LEE genes (115). This sugar-dependent regulation is achieved through two transcription factors: KdpE and Cra (115). The glucose concentration in the environment triggers KdpE phosphorylation by the KdpD histidine sensor kinase. Cra, also known as FruR, uses sugar fluctuations to activate or inhibit the expression of its target genes (115, 116). In addition to regulating the expression of the LEE, Cra and KdpE also regulate catabolic and osmotic stress (115, 116). These regulatory mechanisms provide fine tuning that allows the bacterial cells to adapt themselves to environmental fluctuations related to different concentrations of the carbon sources available and change their basic metabolism, as well as their virulence expression.

Fucose is an abundant carbohydrate in the gut. EHEC employs the FusKR system to exploit fucose as a signal and compete against commensal *E. coli* for intestinal colonization. *Bacteroides thetaiotaomicron*, an abundant member of the microbiota, is able to grow in mucin and produces multiple fucosidases that harvest fucose from the mucus, increasing fucose availability in the intestinal lumen. Fucose is sensed by EHEC via the histidine sensor kinase FusK to modulate LEE gene expression and sugar metabolism (28, 117). Hence, EHEC exploits *B. thetaiotaomicron* to promote the expression of its virulence repertoire (28, 118).

The sugar chemical signaling in EHEC is interconnected with the adrenergic sensing system. QseC and QseE sense Epi and activate QseB and QseF, both of which repress the expression of *fusKR*. Another level of interaction occurs through the QseC-

phosphorylated RR KdpE, which directly interacts with Cra during gluconeogenesis to promote virulence gene expression (Fig. 2). To prevent superfluous energy expenditure, chemical signaling guides EHEC through the biogeography of the GI tract (28). The regulation of virulence may exploit available signals and metabolites; many adaptive conditions are employed to promote pathogens against their competitors.

THE ROLE OF OTHER METABOLITES IN BACTERIAL PATHOGENESIS

Ethanolamine (EA) is an abundant source of nitrogen and carbon in the intestine, and it also serves as a signal for pathogenic bacteria, such as EHEC and *S. Typhimurium*, to activate virulence gene expression (119, 120). The resident microbiota does not metabolize EA efficiently; however, EHEC and *S. Typhimurium* both utilize EA as an important nitrogen source to promote their expansion in the gut and as a signal to control virulence gene expression. Several EHEC virulence genes are induced by EA via the expression of the EutR factor, an EA receptor. Genes induced by EA via EutR include genes encoding fimbrial adhesins, flagella, Shiga toxin, and the LEE (95, 121).

APPROACHES TO INTERFERE WITH BACTERIAL PATHOGENESIS

Recent studies on the chemistry of the microbiome are helping to elucidate how bacteria can scavenge compounds for their own benefit (6, 7, 18). Bacteria employ chemical signaling to communicate among themselves upon the availability of favorable or detrimental compounds. Different aspects of this signaling have been shown in the bacterial cell-to-cell communication field (25, 122–124). The advent of novel technologies will help to obtain more detailed information on the intricate microbiota-pathogen-host relationships to directly identify single molecules or more complex compounds present during *in vivo* infections. The first requirement is to identify and functionally study the effects of nutrient and metabolite changes in specific sites (95).

The approach of designing new compounds based on the regulation of virulence was employed to describe the *N*-phenyl-4-(3-phenylthioureido)benzenesulfonamide molecule, named LED209. This compound came from a large library of 150,000 small organic molecules screened to identify antagonists of AI-3, using an LEE1::LacZ expression reporter in EHEC. It was described as preventing the activation of transcriptional factors of EHEC by blocking the expression of LEE virulence genes that are mediated by the activation of AI-3 (57). After extensive structure-function studies, LED209 was shown to be a prodrug highly selective for the QseC sensor kinase, impairing QseC's function by preventing the activation of virulence genes both *in vitro* and during murine infection. LED209 does not interfere with bacterial growth, potentially reducing selective pressure toward drug resistance (102). This strategy may minimize the development of antibiotic resistance, a high concern for the development of new antimicrobials.

Elucidating the function of intestinal metabolites and bacterial chemical signaling systems may promote the discovery of new antivirulence targets; such targets could include uncharacterized membrane proteins and TCSs related to the signaling cascades, without any disruption of basic metabolic pathways. Additional studies are necessary to understand these complex sensing networks in different isolates, since they may have different regulation or functions in pathogens other than those previously studied (25, 94, 125–127). A better knowledge of all intestinal metabolites and the microbial mechanisms for sensing them will contribute to novel therapeutics.

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