The Interplay between the Microbiota and Enterohemorrhagic Escherichia coli

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ABSTRACT The gastrointestinal tract of mammals is home to a plethora of microbial species that comprise the microbiota. The role of the microbiota in human health is at the forefront of science in recent years, because it is now appreciated that this intricate microbe-host association shapes the host’s immune response and physiology. Many diseases are associated with changes in the microbiota, called dysbiosis. Dysbiosis is associated with obesity, metabolic syndromes, inflammatory bowel-disease, inflammatory bowel syndrome, cancer, diabetes, allergies, and autism. The microbiota is largely regarded as a barrier to enteric infections, such as with enterohemorrhagic Escherichia coli (EHEC). However, the interactions between pathogens and the microbiota are largely unknown, as is how these interactions influence the outcome of enteric disease. The microbial composition of the gastrointestinal tract shapes the landscape in which EHEC survives within the host. This organism competes for nutrients derived from the host diet, liberates additional resources from dietary and host sources, and produces signaling molecules sensed by EHEC to direct gene expression. To successfully colonize the recto-anal junction of a ruminant, the EHEC reservoir, or the colon of a human, an accidental host, EHEC must alter its physiology to survive within the host digestive tract. In this article, we explore the classes of molecules produced or modified by the microbiota that appear to be instrumental in governing virulence gene expression of EHEC. We also explore how interaction with different microbiotas influences EHEC infectivity and host interaction.

INTRODUCTION

The human gastrointestinal (GI) tract harbors trillions of bacterial cells belonging to more than 1,000 species (1), and the number of bacterial cells within the GI tract is 10 times higher than the number of human cells within our bodies (2). The GI microbiota plays essential roles in human nutrition, physiology, development, immunity, and behavior, with disruption of the structure and balance of this community leading to dysbiosis and disease (3–5). This fundamental association between host and bacteria relies on chemical signaling and nutrient availability and exchange. It is also clear that this important balance between host and microbiota can be severely disrupted by environmental stimuli. Two of the most common insults on the microbiota that induce dysbiosis are antibiotic treatment and infectious diseases. Both insults can lead to several disease states ranging from autism, to inflammatory bowel disease, to inflammatory bowel syndrome (IBS). It is also noteworthy that stress exacerbates these syndromes (3).

Broad-spectrum antibiotics alter the microbiome by reducing diversity and shifting community composition (6). Although most of the microbiota return after treat-
ment is ceased, some members of this community are lost indefinitely (6). These community shifts cause changes in the metabolic profiles of the intestine, decreasing concentrations of amino acids and short-chain fatty acids (SCFAs) and increasing oligosaccharide levels, suggesting that microbial fermentation of carbohydrates, a fundamental feature of the microbial flora, was disrupted (7–11). Depletion of SCFAs through antibiotic treatment has critical implications in intestinal health and immunity. SCFAs are rapidly absorbed in the colon and provide a preferred energy source to enterocytes, regulating cell proliferation, differentiation, and apoptosis (12, 13), as well as many aspects of intestinal immunity (12, 13). Butyrate is the SCFA with the strongest effect on cell cycle and plays an anti-inflammatory role in the gut. These anti-inflammatory properties are beneficial because they prevent overinflammation and carcinogenesis (14). Butyrate acts by inducing prostaglandin E₂ (15). It is also worth mentioning that in Crohn’s disease (an inflammatory bowel disease pattern) there are decreased levels of prostaglandin E₂ and butyrate (16), again suggesting that disruption of the metabolome by dysbiosis has important consequences in GI tract health. Another fundamental impact of dysbiosis in the metabolome is disruption of carbohydrate fermentation. Primary fermenters such as Bacteroides spp. are the gateway for the entrance of carbohydrates in the network of syntrophic links in the microbiota, with Bacteroides spp., a prominent glycophagic species, degrading complex carbohydrates into monosaccharides that can be consumed by other members of the gut microbiota (17).

The second important insult that causes dysbiosis is infection by an invading pathogen. It is known that invading enteric pathogens such as Salmonella spp. and Citrobacter rodentium cause inflammation within the gut that in turn diminishes the overall numbers of bacteria in the microbiota, acting as a competition advantage to the pathogen (18, 19). Additionally, infection with C. rodentium also causes significant changes in the structure of the microbial community, decreasing the number of anaerobes and increasing the numbers of Gammaproteobacteria (18). C. rodentium is a murine pathogen that models the enteric infection of the human pathogen enterohemorrhagic Escherichia coli (EHEC). The most compelling evidence for the involvement of the microbiota in disease states comes from IBS, with the chief support coming from data on postinfection IBS, whereby IBS ensues following an episode of bacteriological gastroenteritis (3). The highest supported incidence of postinfection IBS is related to the Walkerton outbreak, when postinfection IBS developed in the majority of individuals after an EHEC infection (20).

**MICROBIOTA/EHEC QUORUM-SENSING SIGNALING INTERACTIONS WITHIN THE INTESTINE**

EHEC colonizes the human colon, where it has to interact and successfully compete with the microbiota to find a colonization niche. The first appreciation of EHEC-microbiota interactions on EHEC pathogenesis came from the observation that EHEC employs quorum-sensing (QS) signaling to regulate expression of its virulence genes (21). QS is a bacterial cell-to-cell signaling mechanism through which bacterial cells assess the density of their population. These bacteria secrete hormone-like compounds, usually referred to as auto-inducers. When these auto-inducers reach a threshold concentration, they interact with transcriptional regulators to drive bacterial gene expression (22). Because EHEC infection requires a low infectious dose, estimated to be between 50 and 100 CFU, it was deemed unlikely that EHEC was responding to self-produced signals upon infection of the host, and it was proposed that EHEC was sensing auto-inducers produced by the GI microbiota (21). The identity of this QS signal was initially a matter of debate. QS was first described in the regulation of bioluminescence in Vibrio fischeri and Vibrio harveyi (23, 24). The luciferase operon in V. fischeri is regulated by two proteins, LuxI, which is responsible for the production of the acyl-homoserine-lactone (AHL) autoinducer, and LuxR, which is activated by this autoinducer to increase transcription of the luciferase operon (25, 26). Since this initial description, homologs of LuxR-LuxI have been identified in other gram-negative bacteria, and in all of these LuxR-LuxI systems, the bacteria produce an AHL, which binds to the LuxR protein and regulates the transcription of several genes involved in a variety of phenotypes (27–29). E. coli has a LuxR homolog, SdiA (30), but does not have a luxI gene and does not produce AHLS (31, 32). Because of these features it was considered unlikely for many years that EHEC had any functional QS systems. However, the discovery of the autoinducer-2 (AI-2) system, present in both Gram-positive and Gram-negative bacteria (including EHEC), suggested that other QS systems, evolved to promote interspecies communication within bacterial populations, may be employed by EHEC to regulate its virulence repertoire within the GI tract (21, 33).

AI-2 was originally identified in V. harveyi as an inducer of cell density-dependent bioluminescence. AI-2 is synthesized from S-adenosylmethionine in three enzymatic steps, the last of which is catalyzed by LuxS producing 4,5-dihydroxy-2,3-pentanedione that
spontaneously cyclizes to form (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (AI-2) (Fig. 1) (34). LuxS is highly conserved, with homologs found in a wide variety of pathogenic and commensal bacteria within *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Human stool samples and *in vitro* propagated commensal bacteria from healthy individuals are capable of inducing luminescence from *V. harveyi*, suggesting that AI-2 is produced within the GI tract (35). EHEC encodes a LuxS homolog, and preconditioned media from this strain are capable of inducing luminescence from *V. harveyi* in a luxS-dependent manner (36), suggesting that EHEC is capable of producing AI-2. Supernatants of EHEC grown to late exponential phase contain a signal capable of inducing transcription of many EHEC virulence genes, including the locus of enterocyte effacement (LEE) that encodes a type III secretion system (T3SS) essential for EHEC to attach to and efface enterocytes and promote disease (37, 38), the flagellum regulon, and expression of Shiga toxin (35, 39). Purified and synthetic AI-2 was unable to govern LEE and flagella expression (40), suggesting the existence of an additional class of autoinducer (AI-3). The identity of AI-3 is not yet known, but it is methanol soluble (35), likely tyrosine derived (36), and shares signaling mechanisms with host-derived epinephrine and norepinephrine (Fig. 1). AI-3 signal is produced by a variety of enterobacterial species, including pathogenic strains and normal flora such as *Enterobacter cloacae* (36), and is also present in the stool of humans (35). The reliance of a functional luxS gene in the presence of AI-3 in EHEC was not due to LuxS being involved in the synthesis of AI-3, but to a metabolic shift that occurs in a luxS mutant (LuxS is involved in the central methyl cycle in bacterial cells) that leads to decreased AI-3 production (36).

**FIGURE 1** Structure of known quorum-sensing ligands. *N*-hexanoyl-l-homoserine lactone (C6-HSL) (A) and *N*-(3-Oxo-octanoyl)-l-homoserine lactone (3-oxo-C8-HSL) (B) stabilize SdiA, which can suppress T3S. (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (AI-2) (C) appears to have a surprisingly modest effect on virulence. QseC responds to host-derived epinephrine (D) and is antagonized by synthetic LED209 (E), perhaps yielding clues to the identity of AI-3. Indole (F) is a tryptophan-derived metabolite that influences motility and type III secretion. Mucin degradation releases fucose (G), which activates the FusKR two-component system to downregulate T3S. SCFAs, including acetate (H) and butyrate (I), induce motility of EHEC, while only butyrate induces T3S via Lrp activity. doi:10.1128/Microbiolspec.EHEC-0015-2013.f1
**Interkingdom Chemical Signaling in EHEC-Host Interactions**

The AI-3 QS signaling system intersects with the host adrenergic signaling system (epinephrine/norepinephrine [NE] hormones) (40). In fact, this intersection occurs at a biochemical level, with the same bacterial receptor, the membrane-bound histidine sensor kinase (HK) QseC, sensing the bacterial signal AI-3 and the host signals epinephrine and/or NE (41). Both epinephrine and NE are present in the GI tract. NE is synthesized within the adrenergic neurons within the enteric nervous system (42). Epinephrine is synthesized in the central nervous system and in the adrenal medulla; it acts in a systemic manner after being released into the bloodstream, thereby reaching the intestine (43). Both hormones modulate intestinal smooth muscle contraction, submucosal blood flow, and chloride and potassium secretion in the intestine (44). Epinephrine and NE are recognized by adrenergic receptors in mammalian cells; Freddolino et al. (45) reported that the ligand-binding sites for epinephrine and NE in a human adrenergic receptor are similar. Extensive evidence indicates that both epinephrine and NE are recognized by the same receptors and play important biological roles in the human GI tract.

The AI-3/epinephrine/NE interkingdom signaling cascade activates expression of virulence genes in EHEC (39, 40, 46, 47). The host hormones epinephrine/NE are specifically sensed by two HKs: QseC and QseE, which are the first bacterial adrenergic receptors identified (46, 48). QseE is downstream of QseC in this signaling cascade, given that transcription of qseE is activated through QseC (49). In addition to sensing these host hormones, QseC also senses the bacterial signal AI-3 (46). The QseC sensor is the first example of a receptor for both a bacterial and a host signal, integrating bacterial-host signaling at the biochemical level. QseE, however, does not sense AI-3, thereby discriminating between host- and bacteria-derived signals (49). Upon sensing their respective signals, QseC and QseE activate virulence gene expression and pathogenesis in vitro and in vivo in EHEC (46, 48, 50). QseC and QseB constitute a cognate two-component system, and the qseBC genes are cotranscribed in an operon that is also autoregulated (51). QseC transfers its phosphate to three response regulators (RRs): its cognate RR QseB and the noncognate QseF and KdpE RRs (52). QseE only transfers its phosphate to its cognate RR QseF (53). The concerted action of these RRs activates the EHEC virulence repertoire (Fig. 2).

QseC facilitates both phosphorylation and dephosphorylation of QseB. Unphosphorylated QseB binds upstream of the flhDC promoter (FlhDC operons are the master regulators of the flagella regulon) and inhibits transcription of these master activators of motility, whereas phosphorylated QseB induces expression (52, 54). QseF indirectly stimulates the expression of espFU/tccP (49), a critical non-LEE-encoded effector required for actin recruitment at the site of pedestal formation. Activation of QseC induces Shiga toxin expression (50) and this is dependent on QseF (52). KdpE functions as an activator of LEE1 (LEE1 encodes the Let activator of all LEE genes [55]) expression (52) by direct binding upstream of the distal LEE1 promoter (56) (Fig. 2). An important note is that the QseC signaling cascade can be inhibited by the antivirulence drug LED209, preventing activation of the EHEC virulence repertoire (50).

**Nutrient Signaling in EHEC-Microbiota Interactions and Virulence Regulation**

The mammalian GI tract harbors trillions of indigenous bacteria whose coexistence relies on the ability of each member to use one or a few limiting resources (1). Invading pathogens have to compete with the microbiota for these resources to establish colonization. These pathogens tend to be aggressive and greedy in search of a colonization niche, and achieve this purpose by precisely coordinating expression of an arsenal of virulence genes. Linking carbon and nitrogen metabolism to the precise coordination of virulence expression is a key step in the adaptation of pathogens toward recognizing suitable sites for colonization, and a means to tip the scale in the tug-of-war between pathogens and the microbiota.

EHEC is no stranger to this “struggle” for nutrients. In addition to purpose-built signaling molecules like the autoinducers, the interplay between the nutrient requirements of normal flora and EHEC is important in determining virulence. Glucose and other monomeric dietary sugars are ideal carbon sources for EHEC, but these molecules are scarce in the lower GI tract. The proximal small intestine is the site of host absorption of simple sugars, whereas the distal small intestine houses a vast population of commensal bacteria scavenging for free sugars. As a result, the colonic home of EHEC is a gluconeogenic environment. Njoroge and colleagues uncovered the importance of glucose availability in regulating T3SS by EHEC (56). Stemming from the observation that high-glucose growth media suppressed type III secretion (T3S) while low-glucose conditions induced LEE expression, the authors uncovered a role for the catabolite repressor/activator protein (Cra) in ler regulation. Indeed, a cra-deficient mutant of EHEC exhibited diminished attaching and effacing (A/E) lesion formation, LEE1 promoter activity, transcript levels,
and EspA secretion under low-glucose conditions. However, under glucose-rich conditions no effect was seen. A Cra-binding site was identified upstream of the distal LEE1 promoter, and binding to this site in an electrophoretic mobility shift assay could be prevented by the inclusion of the glycolytic intermediates fructose-1-phosphate or fructose-1,6-bisphosphate. Cra was found to directly interact with the RR KdpE, previously found to positively regulate LEE1 (52). Interestingly, KdpE-dependent LEE regulation was also found to be in effect only in low-glucose conditions, and KdpE binding in vitro to the LEE1 promoter was diminished by phosphorylation (56). Presumably, under high-glucose conditions, KdpE is phosphorylated by its cognate sensor kinase, KdpD, which is activated by the glucose-sensitive IIA^Ntr phosphotransfer system (57). These data suggest that Cra and KdpE act in concert to induce T3S by inducing ler expression and that this control is only active under glucose-limiting conditions, such as those found within the colon.

While a gradient of diet-derived glucose may provide an indicator of progress through the length of the GI tract, spatial regulation of virulence factor expression likely requires EHEC to distinguish luminal from host membrane proximal environments (Fig. 3). A thick layer of goblet cell-derived mucus partitions the bacteria-laden GI lumen from the host enterocytes. The mucous layer is composed of a dense matrix of cross-linked mucin proteins heavily decorated with O-linked glycans. Within the colon, Muc2 represents the dominant species of mucin linked to glycans composed of GalNAc, NANA, GlcNAc, galactose, fucose (Fig. 1), and mannose monosaccharides, listed in terms of decreasing relative abundance (58). Members of the normal flora of the colon express mucolytic enzymes to harvest carbon from this barrier and from dietary polysaccharides. Bacteroides thetaiotaomicron, a well-studied commensal, is capable of metabolizing pectins, starches, fructan, alpha-glucans, and a number of glycans originating...
from host tissues (59). In contrast, *E. coli* is an organism adapted for exploitation of monosaccharides and tricarboxylic acid cycle intermediates liberated from complex carbohydrates by these commensals (61, 62). It is not surprising that mucin-derived carbon-source sensing has been adapted by EHEC to regulate virulence mechanisms.

Pacheco et al. describe a novel two-component system that enables regulation of virulence gene expression and carbon-source choice by EHEC upon sensing fucose (62). The *fusKR* operon is within the genomic O-island 20, found only in O55:H7 descendant strains and within *C. rodentium*. FusK is a transmembrane HK that autophosphorylates at a histidine residue and transfers the phospho-group to an aspartate of FusR to regulate DNA binding. The authors observe that the FusKR two-component system functions as a repressor of T3S. Knockout of either *fusK* or *fusR* results in increased A/E lesion formation in a cell culture infection model, increased LEE transcript levels, and higher levels of EspB in culture supernatants compared to wild type. Given that FusK shares sequence homology with UhpB, a glucose-6-phosphate sensor kinase, the authors explored whether FusK was responsive to sugar monomers and found that fucose, but not other sugars, was sufficient to induce autophosphorylation of the kinase. Expression of *LEE1* is diminished in wild-type EHEC grown with fucose as the sole carbon source when compared with glucose; however, no difference is seen in *fusK*-deficient EHEC. Similarly, mucin was found to diminish *LEE1* expression when EHEC was grown in the presence of *B. thetaiotaomicron*, which expresses fucosidases capable of liberating fucose from mucin. The effect was not seen with cocultures grown in the presence of fucose rather than mucin. Altogether, these data suggest that fucose serves as a signal to downregulate T3S via FusKR when EHEC is in the lumen.

As the mucous barrier limits the approach of mucolytic commensals toward the epithelial surface, fucose liberation occurs in the colonic lumen, but not in close contact with the intestinal epithelium. LEE expression within the lumen is not appropriate for EHEC; therefore, a luminal signal to decrease T3S would serve to ensure efficient resource expenditure. While in the lumen, fucose-FusK signaling satisfies this need. However, upon reaching the epithelium, FusKR would be detrimental to EHEC as it would inhibit establishment of pedestals. EHEC has used the interkingdom signaling systems of QseBC and QseEF to alleviate this issue while at the epithelial surface. QseB directly binds to and inhibits transcription of the *fusKR* operon, while QseF likely induces expression of a *fusKR* repressor (62). Additionally, EHEC also uses ethanolamine, a vast source of nitrogen within the intestine that is present within membranes, to regulate LEE and Shiga toxin expression (63).
Intriguingly, FusKR was also found to downregulate a putative fucose transporter protein encoded near fusKR. Deletion of this major facilitator superfamily protein locus, z0461, dramatically diminishes the expression of genes involved in fucose utilization and delays growth in media containing fucose as the sole carbon source. On the surface, this observation represents a paradox in that EHEC senses fucose, a prime carbon source, and yet diminishes its own capacity to use fucose. This can perhaps be explained by observations concerning carbon-source preference in physiological settings of bovine small intestine contents (64) and a murine infection model (65). Though fucose utilization contributes to EHEC growth within bovine small intestinal contents, deficiencies within this pathway are not as dramatic as those seen in galactose or mannose utilization pathways (64). Analysis of mouse colonization of mutants of EDL933 or MG1655 demonstrates that fucose is used by both virulent and avirulent strains of E. coli, whereas galactose and mannose are primarily the forte of EHEC and dispensable for MG1655 (63). Therefore, downregulation of fucose utilization systems may be a mechanism of EHEC to avoid carbon-source overlap with commensal E. coli and thus avoid direct competition. This possibility is further corroborated by the work of Kamada et al. (66); these authors observed that expression of virulence genes by the EHEC surrogate murine model C. rodentium is activated at the onset of infection, and at later time points, C. rodentium infection backfires, triggering a bloom of Gammaproteobacteria that effectively compete with C. rodentium for carbon sources. This bloom of Gammaproteobacteria upon C. rodentium infection has also been previously reported by Lupp et al. (18), further suggesting that competition for carbon sources with the microbiota plays an important role in EHEC clearance from the GI tract.

Fermentation of starches within the colon is primarily mediated by Firmicutes, such as Faecalibacterium prausnitzii and Eubacterium rectale, and also Bacteroides spp. to produce SCFAs. The importance of the microbiota to SCFA production is clear from the very low level of these metabolites in germfree animals (67). Acetate, propionate, and butyrate (Fig. 1) dominate the SCFA population and contribute broadly to host physiology (68). SCFAs are present in the colon in millimolar concentrations and exist in much lower concentrations in the upper GI tract. Thus, they represent an excellent indicator of arrival within the large intestine in much the same way that abundant glucose is indicative of localization within the small intestine. As such, EHEC uses these molecules as a cue to govern expression of virulence genes.

Butyrate, but not acetate or propionate, is capable of inducing T3S from the Sakai strain of EHEC (69); 20 mM butyrate increases adhesion to Caco-2 cells 10-fold over control and facilitates the formation of microcolonies (70). Leucine is able to induce LEE4 and LEE5 protein expression similarly to butyrate (69). Both leucine- and butyrate-driven expression of the LEE is dependent on expression of the leucine-responsive protein (Lrp) transcription factor. Given the similarities in the structure of leucine and butyrate and the observation that the M124R leucine-insensitive mutation to Lrp eliminates butyrate-induced LEE expression, it is likely that butyrate is directly sensed by Lrp. In addition to requiring Lrp, butyrate-induced T3S requires Ler and the ler activator PchA. In fact, the promoter of pchA cannot be substituted without abolishing the butyrate effect, implying a cascade of events: butyrate binds Lrp, which then activates transcription of pchA, which in turn activates transcription of the LEE1-encoded ler.

Acetate, propionate, and butyrate increase motility of the Sakai strain (70). Synthesis of the flagellin subunit, FlIC, is increased, and the frequency of flagellated bacteria increases upon exposure to these SCFAs. Two mechanisms of induction are in play: activation of the class 1 flhDC promoter by butyrate and induction of class 2 flgN by acetate, propionate, and butyrate. Butyrate-induced flhDC expression requires lrp, whereas lrp is dispensable for FlgN accumulation.

SCFAs heavily influence the colonic epithelium by serving as an energy source for host use, promoting nutrient absorption, and regulating cell differentiation. Zumbrun and colleagues recently demonstrated that butyrate increases globotriaosylceramide expression on human colonic epithelial cells (71). Mice fed a high-fiber diet, which increases the concentration of butyrate within the intestinal lumen, express higher globotriaosylceramide levels within the intestinal epithelium and in kidneys. When challenged with EHEC, mice fed a high-fiber diet experience a higher pathogenic burden, significant weight loss, and decreased survival compared to animals fed a low-fiber diet. Interestingly, a high-fiber diet diminishes the frequency of Escherichia spp. within the gut while increasing total flora levels. These results suggest that a high-fiber diet, via increased butyrate levels, may increase susceptibility of animals to Shiga toxin released from EHEC by promoting the expression of the receptor necessary for toxicity. Additionally, a high-fiber diet may reduce competition faced by EHEC during infection by reducing levels of normal E. coli
flora while simultaneously increasing the prevalence of other species that may benefit EHEC.

Another important aspect of the role of SCFAs and the outcome of EHEC infections stems from the observation that probiotic strains of *Bifidobacterium longum* that produce high levels of acetate effectively prevent Shiga toxin translocation through the intestinal epithelium. The authors propose that acetate produced by these probiotic bacteria improves intestinal defense and protects the host against lethal infection (72).

The interplay of microbiota and host diet likely creates a multidimensional gradient of metabolites within the GI tract of mammals. EHEC adapted to colonization of the lower intestinal tract uses these gradients to regulate expression of complex, metabolically expensive virulence systems such as the LEE-encoded T3SS to maximize fitness within an animal host. Under such a model, localization within the colon is indicated by increased concentrations of fermentation products, such as butyrate, and increasingly gluconeogenic conditions, which promote expression of the LEE and, thus, attachment as is appropriate. A luminal-epithelial axis is created within the colon by the presence of luminal sugars liberated from carbohydrates, such as fucose derived from mucin, that suppress LEE expression until the epithelium is within reach.

**MICROBIOTA COMPOSITION AND SUSCEPTIBILITY TO EHEC INFECTIONS**

Within the scope of the complex associations between EHEC and different members of the GI microbiota, a burning question is to how much microbiota differential composition contributes to host resistance to EHEC infections? EHEC infections can vary in their degree of severity, ranging from watery diarrhea, to severe bloody diarrhea, to hemolytic-uremic syndrome. It has been reported that antibiotic treatment that alters the microbial composition of the GI microbiota may increase susceptibility to GI infection by *C. rodentium* (73). It has also been reported that microbiota transplantation from susceptible mice to mice resistant to *C. rodentium* infection also increased susceptibility of resistant mice to this pathogen. In the opposite experiment, transplantation of microbiota from resistant mice to susceptible mice was protective (74). Additionally, the combination of certain microbiota members with different metabolites can result in different outcomes on EHEC virulence gene expression. Fucose released from the mucus by *B. thetaiotaomicron* decreases LEE gene expression (62), while under gluconeogenic conditions in the absence of fucose, *B. thetaiotaomicron* promotes LEE gene expression (56). It has also been reported that expression of Shiga toxin is decreased in the presence of *B. thetaiotaomicron* in Leedle and Hesplee medium (75).

**DIFFERENTIAL SIGNALING SYSTEMS GOVERNING HOST ASSOCIATIONS**

The main environmental reservoir of EHEC is ruminants, and it is estimated that 70 to 80% of cattle herds in the United States are colonized with EHEC (76–81). EHEC is an example of a bacterium that behaves as a commensal or a pathogen, depending on its host. EHEC is a commensal in the GI tract of adult cattle but is a human pathogen (82). EHEC colonizes the large intestine of humans, forming A/E lesion, thought to be largely responsible for promoting disease (82). The genes for A/E lesion formation are encoded within the LEE (82). The LEE and A/E lesion formation are also necessary for EHEC colonization of the recto-anal junction (RAJ) of cattle, facilitating shedding of this pathogen in the environment (83). In addition to the LEE, EHEC uses the glutamate decarboxylase (*gad*) acid resistance system to survive passage through the acidic stomachs of these animals to reach its site of colonization, the RAJ (84). *SdiA* is a regulator that senses AHL QS signaling molecules and aids EHEC survival and colonization of the bovine GI tract. AHLs are prominent within cattle rumen but absent in the other sections of the GI tract. Through *SdiA*, transcription of the LEE genes is decreased by rumen AHLs, while transcription of the *gad* acid-resistant system is increased. Expression of the LEE in the rumen would be an unnecessary energy burden for EHEC in this GI compartment. However, in preparation for the acidic distal stomachs, the EHEC *gad* is activated in the rumen. *SdiA-AHL* signaling aids EHEC in gauging these environments and modulates gene expression toward adaptation to a commensal lifestyle in cattle. Consequently, an *sdiA* mutant is deficient for cattle colonization (85, 86) (Fig. 4).

AHLs are synthesized from S-adenosylmethionine and an acylated acyl carrier protein by AHL synthases, such as LuxI of *V. fischeri* or RhlI of *Pseudomonas aeruginosa* (87). AHLs are sensed by a class of unstable transcription factors exemplified by LuxR of *V. fischeri*. LuxR homologs consist of an N-terminal autoinducer binding domain and a C-terminal helix-turn-helix motif. AHL binding promotes LuxR folding and dimerization to allow for binding to a target sequence within a promoter to regulate gene expression. Acyl chain length and structure provide specificity for AHL sensing by different
species. *E. coli* does not express any known AHL synthase but does encode for a *luxR* homolog, *sdiA*.

Initial studies aimed at understanding the role of *sdiA* in virulence of EHEC observed that overexpression of SdiA diminished production of LEE4 and LEE5 encoded proteins, as well as decreasing flagellin expression and soft agar motility (88). Like other LuxR homologs, SdiA tends to fold poorly in the absence of exogenous AHLs and accumulates in inclusion bodies. Overexpression of SdiA likely results in a small, transient population of soluble molecules that is capable of affecting transcription. Indeed, transcriptional analysis of *sdiA*-deficient EHEC reveals that the glutamate-dependent acid resistance (*gad*) genes are activated by SdiA independently of exogenous AHL activity, although AHLs enhance this activation (85). In contrast, SdiA regulation of the LEE T3SS is AHL dependent. SdiA-deficient EHEC has no change in LEE expression relative to wild type in the absence of AHL. Exogenous oxo-C6-homoserine lactone (Fig. 1) diminishes LEE1 transcription and EspA secretion in an SdiA-dependent manner. SdiA stabilized by exogenous AHLs directly binds to the *LEE1* promoter to repress *ler* transcription and diminishes T3S through this action (85).

Nuclear magnetic resonance structural analysis of SdiA of *E. coli* has demonstrated that the protein is capable of fruitfully interacting with at least three additional AHLs: C8-HSL, oxo-C8-HSL (Fig. 1), and C6-HSL (89). This, combined with the lack of endogenous AHLs from *E. coli*, implies that SdiA likely functions as a sensor for an array of foreign AHLs found within the surroundings of *E. coli*. In support of this notion, AHLs have been found within the rumen contents of cattle fed both grain and forage diets (86, 90, 91). However, chemical analysis of lower GI tract contents from cattle and nonruminant animals has not revealed the presence of AHLs, which may be due to inadequate sensitivity of the methods used, alkaline instability of AHLs, or potentially the existence of exotic homoserine lactones or non-AHL small molecules that may influence SdiA function (40).

These reports highlight how different chemical signaling systems can be employed by bacteria to adapt to either pathogenic or commensal lifestyles in different hosts and that this signaling system aids this human pathogen to adapt to a commensal lifestyle in cattle, its main reservoir.

**CONCLUDING REMARKS**

The increasing knowledge of the essential roles of the microbiota in human health is opening many avenues of research to understand differential host susceptibility to infectious diseases. These studies are fundamental for enteric pathogens, which inhabit a complex and dynamic environment. It is fascinating to envision how such few EHEC organisms (circa 50 CFU) efficiently manage to establish themselves in the host and cause disease. It is becoming clear that EHEC is very crafty at reading many cues provided by both the host and the microbiota and rapidly adapting its virulence program toward successful host infection. We are also at the tip of an iceberg in determining how different microbiota enterotypes may determine the severity of EHEC disease. One should also take into consideration that differences in diets and antibiotic regimens, which cause shifts in the composition of the GI microbial flora, may also influence the outcome of EHEC disease.
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The Interplay between the Microbiota and Enterohemorrhagic Escherichia coli


