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Commensal "trail of bread crumbs" provide pathogens with a map to the intestinal landscape

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Abstract

Growth of a microorganism in a host is essential for infection, and bacterial pathogens have evolved to utilize specific metabolites to enhance replication *in vivo*. Now, emerging data demonstrate that pathogens rely on microbiota-derived metabolites as a form of bacterial-bacterial communication to gain information about location within a host and modify virulence gene expression accordingly. Thus, metabolite-sensing is critical for pathogens to establish infection. Here, we highlight recent examples of how the foodborne pathogen enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) exploits microbiota-derived metabolites to recognize the host intestinal environment and control gene expression that results in controlled expression of virulence traits.

Graphical Abstract

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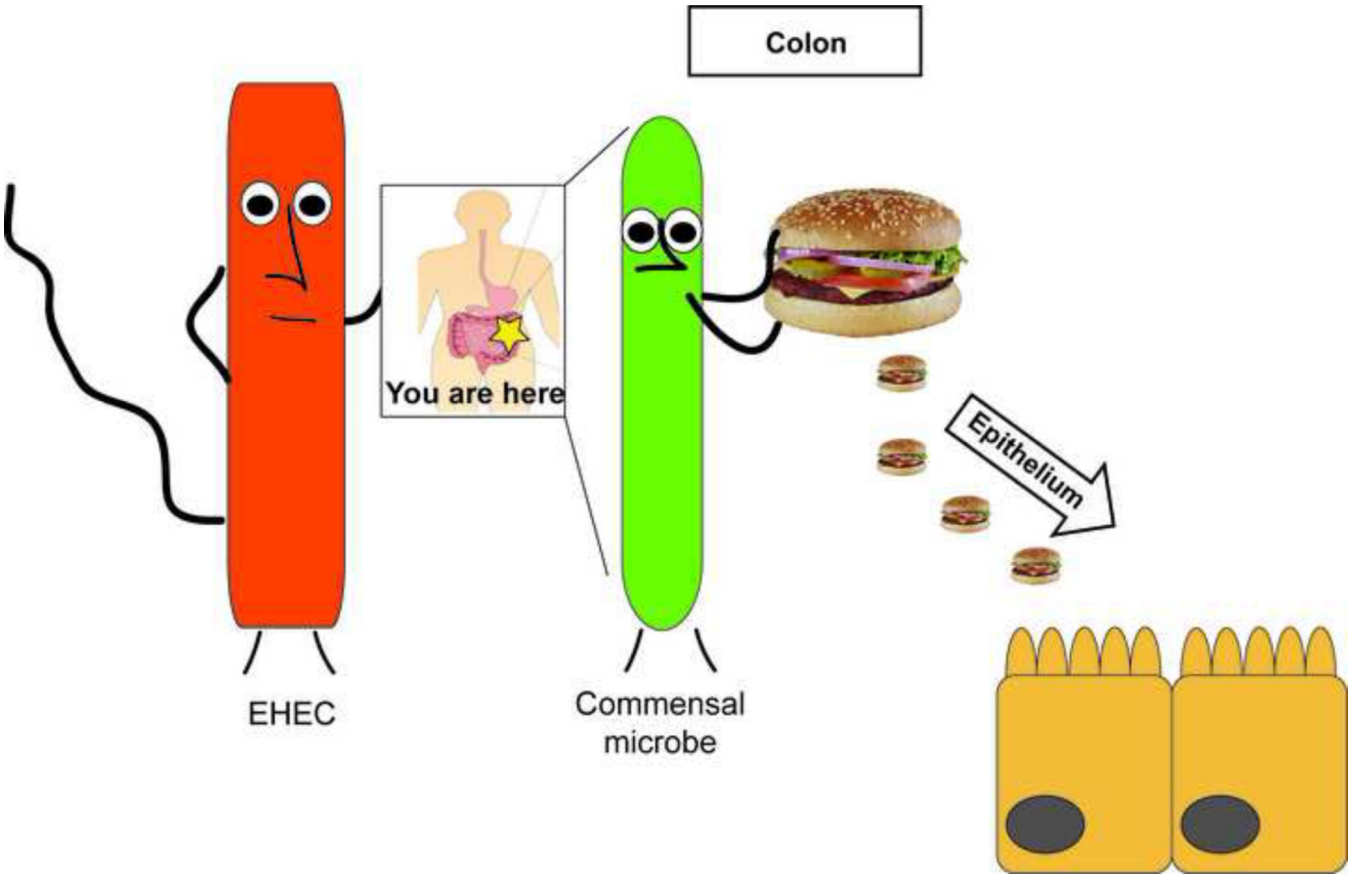

Introduction

The gastrointestinal (GI) tract is inhabited by trillions of commensal bacteria collectively referred to as the resident microbiota that aid in the development of the immune and digestive systems as well as in vitamin and nutrient production [1]. Additionally, the microbiota act as a barrier against infection by invading pathogens through efficiently utilizing nutrients, and thereby limiting the growth of pathogens within the host. This process is called colonization resistance [1]. However, bacterial pathogens have also evolved means of overcoming colonization resistance by utilizing non-competitive substrates for growth and/or exploiting dysbiosis, which is a disturbance in the microbiota that commonly results from antibiotic use [2, 3]. In addition to selectively using metabolites for *in vivo* replication, emerging data have revealed that microbiota-derived metabolites form the basis for communicating spatiotemporal information to bacterial pathogens. This is used to adapt to distinct host niches and precisely control the expression of virulence traits.

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 causes major outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS) worldwide. EHEC colonizes the large intestine and forms attaching and effacing (AE) lesions on the intestinal surface. AE lesions are characterized by intimate attachment of EHEC to enterocytes and effacement of the microvilli [4–6]. The locus of effacement (LEE) pathogenicity island encodes a type

three secretion system and most of the effectors required for AE lesions [7], as well as the transcription factor Ler that is the master regulator of the LEE [8]. EHEC also produces a Shiga toxin that causes HUS and can lead to fatal outcomes associated with EHEC infections [9]. Significantly, EHEC has a very low infectious dose (as low as 50 colony forming units) [10], which is a major factor contributing to outbreaks, and indicates that EHEC has evolved mechanisms to outcompete commensal bacteria for nutrients and rapidly respond to environmental cues to coordinate expression of virulence traits. In this review, we highlight recent, notable examples of commensal-derived metabolites that EHEC senses as signals of the intestinal environment and the corresponding regulatory cascades that modulate expression of the LEE and Shiga toxin, as well as other virulence factors important for host colonization and infection.

Ethanolamine

Ethanolamine is a component of phosphatidylethanolamine, an abundant lipid in eukaryotic and bacterial cell membranes. As a component of phosphatidylethanolamine and other modified lipid molecules, ethanolamine is an important signaling molecule and influences immunomodulation, cell division, nutritional intake, and energy balance [11–14]. The exfoliation of enterocytes as well as the turnover of bacterial cells releases an abundant and replenished supply of ethanolamine in the GI tract. Although ethanolamine can serve as a carbon and/or nitrogen source for bacteria, the resident microbiota do not readily metabolize ethanolamine [15]. Thus, intestinal pathogens, including EHEC, utilize ethanolamine to sidestep nutritional competition and enhance growth during infection [3, 15–17].

Significantly, bacterial pathogens respond to ethanolamine as a signaling molecule to activate virulence gene expression [18–20••] (Fig. 1). In EHEC, ethanolamine activates the expression of genes critical for colonization of the GI tract, including fimbrial adhesins and the LEE, as well as genes encoding Shiga toxin [19••, 20]. Fimbrial adhesins are extracellular proteinaceous structures that mediate binding of bacteria to surfaces, including host epithelial cells. EHEC encodes 16 distinct fimbrial loci [21, 22], and these fimbriae may be important for initial adherence to enterocytes, that precedes intimate, LEE-dependent adherence [23]. However, the contribution of many fimbrial loci to EHEC pathogenesis has been elusive due to the difficulties of expressing fimbrial genes *in vitro* [24]. Thus, the finding that the biologically relevant molecule ethanolamine promotes expression of EHEC fimbriae suggests that these fimbriae play a role in the ability of EHEC to establish infection. Additionally, ethanolamine activates expression of global regulators in EHEC [20], suggesting that ethanolamine plays a central role in integrating multiple cues, to optimize timing of virulence gene expression.

EHEC and other members of the *Enterobacteriaceae* carry the ethanolamine utilization operon, which contains 17 genes that encodes for the transport and breakdown of ethanolamine [25, 26]. This operon also encodes EutR, which belongs to the AraC/XylS family of transcriptional regulators. EutR directly senses ethanolamine to promote *eut* transcription [20, 27]. Biochemical studies revealed that EutR directly regulates LEE expression by binding to the *ler* promoter [28•], and genetic data suggest that EutR also regulates a subset of EHEC fimbriae [19]. Importantly, EutR-dependent virulence gene

regulation is independent of ethanolamine metabolism, as a deletion of the ethanolamine catalytic enzymes did not affect the ability of EHEC to sense ethanolamine and activate expression of the LEE and Shiga toxin [20]. Genetic data also indicate that EHEC encodes a second ethanolamine sensor, as a *eutR* deletion strain of EHEC is able to promote gene expression in response to ethanolamine [20]. These findings suggest that ethanolamine is a critical signal for EHEC to recognize the host intestinal environment.

Microbiota-liberated sugars

The intestinal epithelium is covered by stratified layers of mucus. In addition to regulating water, vitamin, mineral, and electrolyte absorption, the mucus serves as a physical and chemical barrier separating the resident microbiota and epithelium [29]. Mucus is composed of glycosylated proteins, called mucins, antimicrobial peptides, enzymes, as well as monosaccharides, including fucose [30, 31]. Some members of the resident microbiota, such as *Bacteroides thetaiotamicron* (*B. theta*), contain fucosidases that cleave fucose from host mucin, which leads to fucose availability in the GI tract [30, 32, 33].

EHEC uses a two-component system FusKR to sense luminal fucose and repress LEE expression [34••] (Fig. 1). Two-component systems are composed of a histidine kinase and a response regulator. The histidine kinase phosphorylates in response to a particular environmental stimulus and then transfers the phosphate to a response regulator that typically binds DNA to promote or repress gene transcription [35]. FusK is the histidine kinase that senses fucose and relays this information to the response regulator FusR [34]. FusR directly represses LEE expression, which results in a corresponding decrease in AE lesion formation [34]. *In vivo* studies demonstrated that FusKR enhances EHEC colonization of the GI tract [34] because it functions to decrease unnecessary energy expenditure by repressing the expression of the LEE virulence genes in the lumen where fucose is abundant and expression of the LEE-encoded type three secretion system and effectors would be unproductive. When EHEC reaches the epithelium FusKR is no longer active due to lower fucose concentrations as well as transcriptional repression of *fusK* and *fusR* by the QseBC two-component system that senses epinephrine and norepinephrine [34, 36].

Short chain fatty acids

Short chain fatty acids (SCFAs), which include acetate, butyrate, propionate, and succinate [37–41], are a subset of fatty acids that are produced by the gut microbiota during the fermentation of partially digestible and nondigestible polysaccharides [42]. The diet also provides a source of SFCAs; however, the anaerobic members of the microbiota are largely responsible for the generation of SFCAs, as significantly lower amounts of SCFAs are detected in the intestines of germ-free mice compared to conventionally colonized mice [43]. SCFAs play important roles in host physiology by serving as a major energy source for enterocytes, regulating diverse cellular processes, and influencing inflammatory signaling and the immune responses [44–47].

The largest concentrations of SCFAs are measured in the proximal colon [42]; therefore, it is not surprising that bacterial pathogens rely on sensing SCFAs as a signal to indicate arrival

at this site. EHEC responds generally to SCFAs to activate expression of genes encoding flagella and motility [48]; however, butyrate, specifically, enhances LEE gene expression and adherence to epithelial cells [49]. In response to butyrate, the leucine-responsive regulatory (Lrp) protein initiates a signaling cascade that promotes expression of *pchA* [49], which encodes a direct activator (PchA) of the LEE [50, 51]. Recent work by Takao *et al*. revealed that butyrate-dependent activation of the LEE is even more complex and also includes the transcription factor LeuO [52•]. Lrp directly promotes expression of *leuO*, which in turn also binds the *ler* promoter to activate expression of the LEE and microcolony formation. Interestingly, LeuO activation of the LEE genes required PchA and both PchA and Ler activated *leuO* expression. This positive feedback mechanism is hypothesized to function in prolonging expression of the LEE [52] (Fig. 1).

EHEC also senses succinate to activate virulence gene expression [53••] (summarized in Fig. 1). Succinate is a major by-product of fermentation by *Bacteroides* species [54], which are a significant component of the resident anaerobic microbiota. EHEC senses succinate through the transcription factor Cra to enhance virulence gene expression and AE lesion formation [53].

The important role of succinate in bacterial virulence was corroborated using a mouse model of infection with *Citrobacter rodentium. C. rodentium* is a murine pathogen that carries the LEE and recapitulates EHEC colonization during infection (recently reviewed in [55]). During *C. rodentium* infection, mice reconstituted with *B. theta* presented with more severe disease manifestations compared to mice that were depleted of the normal microbiota. This was due to increased concentrations of succinate in mice with *B. theta* compared to mice in which *B. theta* was absent [53]. *C. rodentium* carries the *cra* gene (also annotated as *fruR*) [56], which shares 99% homology to EHEC Cra [21, 22] (based on amino acid sequences); therefore, succinate sensing through Cra may be a conserved mechanism that AE pathogens use to gauge gluconeogenic versus glycolytic conditions (in conjunction with fucose sensing) within the intestine [57].

Host-derived metabolites

It should be noted that host metabolites also influence EHEC virulence gene expression and host colonization. For example, host-generated bicarbonate influences EHEC and *C. rodentium* virulence [58–60]. Gastric and duodenal mucosae secrete bicarbonate to the lumen to regulate intestinal pH and maintain homeostasis [61, 62]. In *C. rodentium*, bicarbonate is sensed through the global regulator RegA. RegA belongs to the AraC/XylS family of transcriptional regulators and shares homology to important virulence regulators, including Rns in enterotoxigenic *E. coli*, AggR in enteroaggregative *E. coli*, and PerA in enteropathogenic *E. coli* [59]. Upon sensing bicarbonate, RegA expression and activity is stimulated, which results in activation of the LEE genes, through direct regulation of the LEE-encoded regulator *grlA* [63]. RegA also controls expression of fimbrial and afimbrial adhesins that impact *C. rodentium* adherence to epithelial cells [59, 60]. In addition to bicarbonate, the vitamin biotin and the amino acid D-serine influence LEE expression [64•, 65•]. The small intestine is the main site of biotin absorption, and therefore, biotin concentrations are likely to higher at this site compared to the large intestine [65, 66].

Moreover, D-serine is present in the large intestine, but is most abundant at extraintestinal sites, including in the urinary tract [67, 68]. These high concentrations of biotin and D-serine repress LEE expression, suggesting a model in which biotin and D-serine confer niche specificity by preventing EHEC colonization of sites outside of the large intestine [64, 65]. Altogether, these studies highlight complex regulatory pathways based on host- and bacterial-derived cues to ensure precise and coordinated expression of virulence genes.

Conclusions

Growth within a host is a requisite for bacterial infection, and bacterial pathogens take advantage of non-competitive metabolites and/or host dysbiosis to survive and replicate within a host. However, it is becoming increasingly clear that metabolites contribute to pathogenesis beyond promoting growth: metabolites are important signals that pathogens exploit to recognize specific host niches and appropriately modulate virulence gene expression. Here, we described how EHEC relies on sensing microbiota-derived metabolites to integrate several cues to precisely regulate expression of virulence traits. In addition to uncovering novel and complex signaling pathways, these studies have revealed the physiological conditions that promote expression of uncharacterized virulence factors. Importantly, as research continues to focus on the impact of the microbiota-derived metabolites on host physiology, including resistance and susceptibility to infectious diseases, and for their potential use as probiotic therapies [44, 69–71], it is exceedingly important to better elucidate how microbiota-derived metabolites impact bacterial virulence. A greater understanding of the interaction between bacterial virulence and microbiota-derived metabolites will provide a more comprehensive understanding of the impact of current therapies on host physiology as well as assist in the development of novel treatments for infectious diseases.

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Highlights

- The commensal microbiota communicate to pathogens through the release of nutrients
- Pathogens sense microbiota-derived metabolites as cues to recognize host niches and control gene expression
- Better understanding of metabolite-based signaling pathways is necessary to study bacterial virulence factors and develop novel therapies to treat infectious diseases

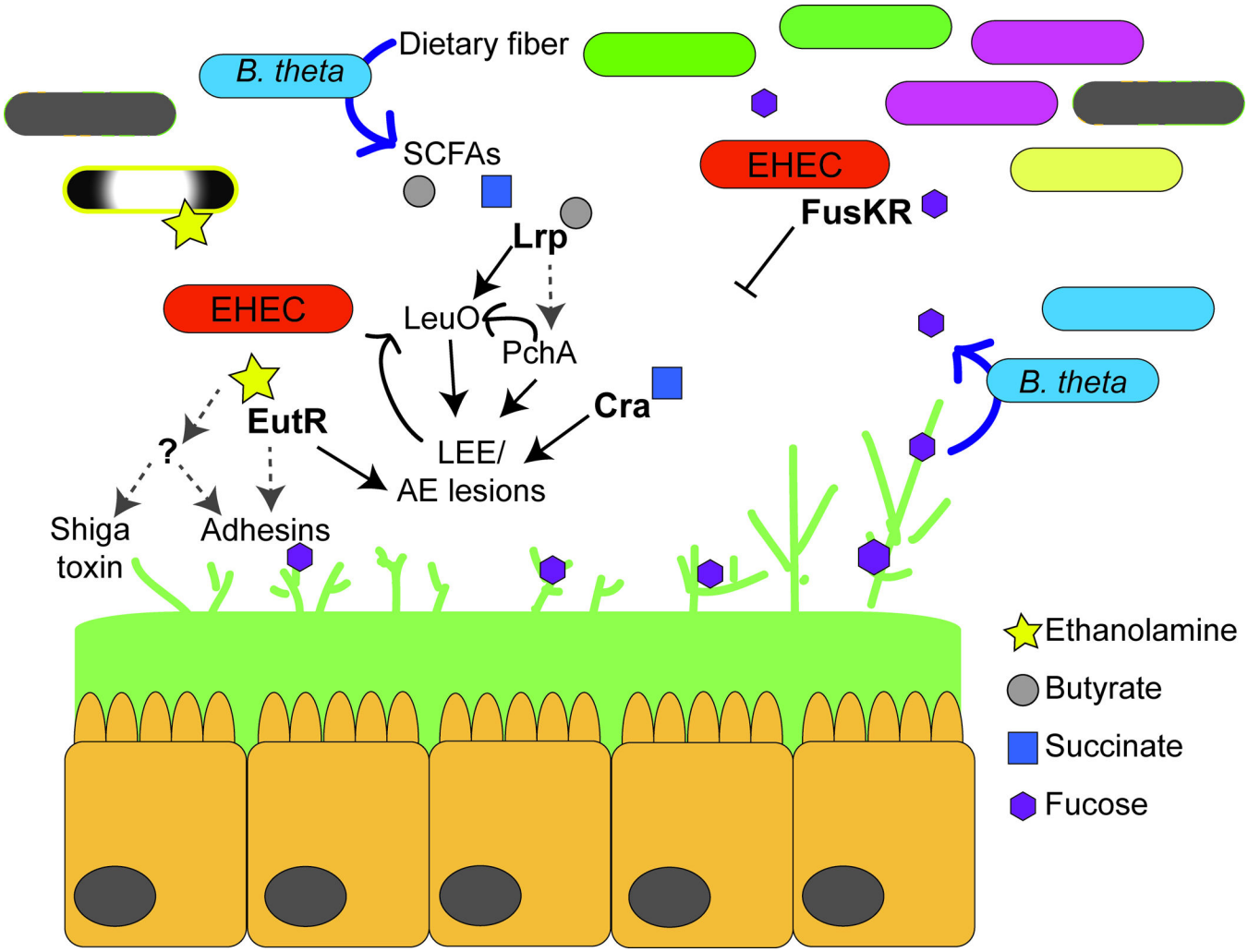

Figure 1.

EHEC responds to microbiota-derived metabolites to activate complex regulatory cascades. EHEC senses ethanolamine, butyrate, and succinate to activate virulence gene expression, whereas the two-component system FusKR represses LEE expression in response to fucose. Ethanolamine is sensed through EutR as well as an unidentified ethanolamine sensor; butyrate is sensed through the transcription factor Lrp; succinate is sensed by Cra. Black lines indicate direct interaction, and grey hashed arrows indicate indirect regulation. For details, refer to main text.