Microbes and Infection 15 (2013) 759–764

www.elsevier.com/locate/micinf

Review

Nutrient and chemical sensing by intestinal pathogens

Juan D. Hernandez-Doria [a,b], Vanessa Sperandio [a,b,*,1]

[a] Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9048, USA
[b] Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390-9048, USA

Received 19 June 2013; accepted 28 June 2013
Available online 9 July 2013

Abstract

Pathogenic gut bacteria, such as those comprising the Enterobacteriaceae family, have evolved sophisticated virulence mechanisms, including nutrient and chemical sensing, to escape host defense strategies and produce disease. In this review we describe the mechanisms utilized by the enteric pathogen enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 to achieve successful colonization of its mammalian host.

Keywords: Enterobacteriaceae; Signals; Type-III secretion; Two-component system

1. Introduction

The gastrointestinal tract plays home to trillions of bacteria that perform essential functions for the host. Pathogenic bacteria must compete with the commensal bacteria in order to thrive and cause disease [1–4]. Successful colonization of the human gut requires that pathogens be skillful users of virtually any molecule available in the gut. The human gut contains two zones with different nutrient/chemical sources for bacteria: the lumen with stool deposits and an epithelial layer rich in mucins. Human stool is rich in "waste" materials originating from the diet and include polysaccharides (starch, hemicellulose), hormones (cortisol, serotonin), secondary metabolites from microflora fermentation (acetate, butyrate, indole-3-propionate), salts and minerals [5,6]. The colonic mucosal layer is further divided into two layers: a loose outer layer that can be utilized by the gut microflora and a tight inner layer free of bacteria [7,8]. The outer layer is composed primarily of *O*-glycan proteins that can serve as nutrients and potential attachment sites [9].

The diet consumed by the host greatly influences which nutrients are available within the gut, in turn affecting the composition of bacteria within the intestine. Two major phyla dominate the adult gut: the Bacteroidetes and the Firmicutes phyla. The Bacteroidetes phyla contain *Bacteroides* and *Prevotella* species while the Firmicutes contains *Lactobacillus* and *Clostridium* species [10]. Compelling evidence indicates the correlation between human diseases such as obesity and shifts in bacterial populations [11]. An obesity mouse model indicates a strong correlation between higher consumption of a Westernized diet rich in saturated fats and complex oligosaccharides and a shift towards a large number of Firmicutes [12]. A study comparing the composition of the gut microflora between individuals consuming a Westernized diet and individuals ingesting a vegetarian diet indicated that consumption of a Westernized diet increased the *Bacteroides* species in the gut whereas consumption of a vegetarian diet increased the *Prevotella* species [13].

The majority of human gut pathogens belong to the Gamma-Proteobacteria class, including the intestinal pathogen that will be covered in this review: Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7. This pathogen is a Gram negative bacterium that employs a syringe-like type-III secretion system (T3SS) to assist in its colonization of the gut

* Corresponding author. University of Texas Southwestern Medical Center, Department of Microbiology, 5323 Harry Hines Blvd., Dallas, TX 75390-9048, USA. Tel.: +1 214 633 1378; fax: +1 214 648 5905.
E-mail address: vanessa.sperandio@utsouthwestern.edu (V. Sperandio).
[1] For express mail: 6000 Harry Hines Blvd. NL4.140A, Dallas, TX 75235, USA.

1286-4579/$ - see front matter
http://dx.doi.org/10.1016/j.micinf.2013.06.013

by injection of effectors into human epithelial cells that change host signaling pathways [14]. Bacteria sense chemicals and nutrients in their environment and engage many transcriptional regulators, including two-component signaling systems (TCS) to regulate their gene expression [15]. A TCS consists of a histidine kinase (HK) sensor protein that autophosphorylates in response to environmental cues and transfers this phosphate to a response regulator (RR) protein, which is generally a transcription factor. TCSs allow bacteria to control cellular functions and respond to environmental conditions including pH, nutrient availability and metabolic end products [15].

2. Enterohemorrhagic *E. coli* (EHEC) O157:H7

Earlier studies involving pathogenic *E. coli* were designed to determine the role of quorum sensing (QS) in the expression of virulence factors in enterohemorrhagic (EHEC) and enteropathogenic *E. coli* (EPEC) [16]. *E. coli* uses several QS systems, such as the *luxS*/autoinducer-2 (AI-2) [16,17], Autoinducer-3 (AI-3)/epinephrine/norepinephrine [18,19], indole [20], and the LuxR homolog SdiA [21,22] to achieve intercellular signaling. The majority of these signaling systems are involved in interspecies communication, and the AI-3/epinephrine/norepinephrine signaling system is also involved in inter-kingdom communication [19]. EPEC is responsible for causing watery diarrhea in children, while EHEC causes bloody diarrhea and the life-threatening hemolytic-uremic syndrome (HUS). EHEC produces Shiga toxin while EPEC does not. However, both types can cause intestinal lesions known as attaching and effacing (AE) lesions [23]. A pathogenicity island (PAI) called the locus of enterocyte effacement (LEE) encodes a cluster of genes, including those responsible for AE lesions [24]. The LEE encode a T3SS [25], an adhesin (intimin) [26] and its receptor (Tir) [27], and effector proteins [28–32]. The *ler* gene encodes for the master regulator of the LEE genes [23,33–35]. An initial study [16] demonstrated that expression of the LEE in EPEC and EHEC O157:H7 is regulated by QS. In addition to the LEE, flagellar expression and motility, and Shiga toxin expression can also be controlled by QS [36,37]. Initial findings suggested that the bacterial-derived signal AI-2 was essential for LEE expression [16]; however, addition of purified and synthesized AI-2 to *in vitro* cultures did not restore LEE expression in an EHEC O157:H7 *luxS* mutant [17,38]. LuxS catalyzes the final reaction of ribosyl-homocysteine into 4,5-dihydroxy-2,3-pentanedione (DPD) for the synthesis of AI-2 [39,40]; thus, it was hypothesized that another autoinducer molecule must be responsible for controlling virulence factors in EHEC [19]. Interruption of *luxS* affects metabolism and reduces production of another chemical signal, AI-3 [17]. A different QS signal, AI-3 overcame the mutation in *luxS,* activated the LEE, and restored motility.

Many commensal and pathogenic bacteria from the gut produce both AI-2 and AI-3 [19]. One hypothesis is that the AI-3 system might be used by pathogenic strains, like EHEC O157:H7, to alert the bacterium of its arrival to the large intestine and initiate virulence gene expression [16,19]. Clarke [41] demonstrated that AI-3, as well as the host hormones epinephrine and norepinephrine, signal through the TCS QseBC, with QseC directly sensing these three signals, to activate flagella expression. From these findings, it was proposed that bacteria and the host communicate with one another using inter-kingdom signaling [41].

2.1. The autoinducer-3/epinephrine/norepinephrine system coordination of flagella-motility genes using the two component system QseBC in E. coli

The QseBC TCS is composed of the membrane-spanning HK kinase, QseC, and the RR QseB. The QseC sensor kinase contains two domains: a histidine kinase domain and an ATPase domain. QseC senses the signals AI-3, epinephrine, and norepinephrine to coordinate virulence gene expression, including expression of flagella and motility through the flagella master regulator FlhDC [41]. Activation of *flhDC* depends on phosphorylated QseB. The phosphorylated QseB protein binds to two different regions of the *flhDC* promoter: the proximal region (−300 bp to +50 bp) and a distal region (−900 bp to −650 bp). The phospho-QseB binds first to the distal region, then later to the proximal region [42,43]. Transcription of *flhDC* occurs in response to a coordinated process dependent on the intensity of the signal received by QseC. If there is a low input signal, then QseB will not be phosphorylated and the protein will bind to a region between −650 and −300 bp which may result in flagella repression [42,43]. At high levels of input signal, a phosphorylated QseB will bind to both the distal and proximal regions of the *flhDC* operon and flagella is activated [42,43].

2.2. O157:H7 controls LEE expression by employing several chemical and nutrient sensing mechanisms

2.2.1. Cra and KdpE regulation of the LEE

The LEE is arranged into five major operons *LEE1* to *LEE5* and encodes a T3SS that permits the attachment and delivery of effectors proteins into the host cell [24,25]. Bacterial effectors induce rearrangement and accumulation of host actin to form the hallmark AE lesions that cup the bacterium tightly to the intestinal epithelium reviewed by Garmendia et al., [44]. Regulation of LEE expression is primarily led by the locus of enterocyte regulator (Ler) [23,33–35]. Transcriptional activation of *ler* is complex and also includes several TCS that interconnect with each other [33]. QseC does not activate exclusively its cognate RR QseB, but also interacts with other RRs such as KdpE and QseF. KdpE is a transcriptional regulator that together with KdpD, its cognate sensor kinase, is responsible for sensing potassium [45]. Meanwhile, QseF is a RR that forms a TCS with the QseE histidine sensor kinase (HK), which senses the host hormone epinephrine, sulfate and phosphate sources [46]. QseC transfers a phosphate to both KdpE and QseF, which in turn activate expression of the LEE and Shiga toxin, respectively [43] (Fig. 1).