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The possible influence of LuxS in the in vivo virulence of rabbit enteropathogenic *Escherichia coli*

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Abstract

Attaching and effacing (A/E) organisms, such as rabbit enteropathogenic *Escherichia coli* (EPEC), human EPEC or enterohemorrhagic *E. coli* (EHEC) share attaching and effacing phenotype and LEE pathogenicity island responsible for A/E. The present study was undertaken to investigate the impact of the LuxS quorum sensing (QS) signaling system in vitro and in vivo pathogenicity of A/E organisms using rabbit EPEC (rEPEC) strain E22 (O103:H2). Analysis of the bioluminescence indicated abolished production of the QS signal AI-2 by *luxS* mutant (E22Δ*luxS*). Strain E22Δ*lux* also exhibited impaired expression of several normally secreted proteins and reduced adherence to cultured HeLa cells. Complementation of the intact *luxS* gene to E22Δ*luxS* restored secreted protein expression comparable to the WT type but not adherence to HeLa cells. In experimentally infected rabbits, the isogenic *luxS* mutant induced clinical illness and intimate adherence to the intestinal mucosa, albeit to a less extent, comparable to that seen with the parent virulent strain. It is worth noting that reduced fecal bacterial shedding, mucosal adherence and improved cumulative weight gain were seen for the mutant strain complemented with *luxS* when compared to the WT. It appears that the *luxS* gene is not essential for in vivo pathogenicity by rEPEC where exogenous QS signals are present in the gut. The impact of AI-2 provided by multicopy plasmid on bacterial virulence is discussed.

Keywords: Quorum sensing; Autoinducer; LEE; Regulation

1. Introduction

The rabbit enteropathogenic *Escherichia coli* (rEPEC) share with human EPEC, enterohemorrhagic *E. coli* (EHEC) or *Citrobacter rodentium* associated

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with colonic hyperplasia in mice the attaching and effacing (A/E) phenotypes, characterized by close bacterial attachment to the epithelial cells and effacement of microvilli (Nataro and Kaper, 1998). The A/E phenotype is encoded on a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE) (McDaniel et al., 1995). The LEEs from rabbit EPEC (rEPEC) O15:H- (Zhu et al., 2001), human EPEC (strain E2348/69, O127:H6) (Elliott et al., 1998), EHEC (O157:H7) (Perna et al., 1998) and *C. rodentium* (Deng et al., 2001) share a conserved region clustering into five major operons, LEE1-5. The LEE encodes a type III secretion system (T3SS), the LEE-encoded regulator (*ler*), bacterial adhesin intimin, translocated intimin receptor (Tir) and various secreted effector proteins (Elliott et al., 1998).

Ler is a central regulator for the expression of the LEE genes in A/E organisms (Mellies et al., 1999). It has been shown that the quorum sensing (QS) controls the expression of T3SS and protein secretion via the activation of Ler in EPEC and EHEC (Sperandio et al., 1999, 2000, 2001). QS confers the ability to communicate and to alter (regulate) behavior in response to fluctuations in cell population density (Miller and Bassler, 2001). QS-mediated signaling can be achieved through the secretion of low-molecular-weight hormone-like compounds called autoinducers (AIs) into the extracellular milieu (Sperandio et al., 2003). When these AIs reach a designated threshold concentration, they interact with bacterial transcriptional regulators, thereby regulating gene expression. While AI-1 (AHL, acyl-homoserine lactones) appears to be the predominant intra-species quorum signal in gram-negative bacteria, an alternate AI-2, discovered in *Vibrio harveyi* (Miller and Bassler, 2001) is proposed to act as an inter-species cellular communication molecule among both gram-negative and gram-positive species (Vendeville et al., 2005; Xavier and Bassler, 2005). The AI-2 signaling pathway has been linked in diverse bacteria to many phenotypes including bioluminescence, antibiotic resistance, biofilms, plasmid conjugal transfer and pathogenicity (DeLisa et al., 2001; Gonzalez et al., 2006).

EPEC/EHEC use an AI-2 system, a furanosyl borate diester, whose synthesis depends on LuxS (Sperandio et al., 2003). Previous studies demonstrated that QS signaling is an important regulatory mechanism for in vitro LEE gene expression by EPEC and EHEC (Sircili et al., 2004; Sperandio et al., 1999). However, the role of LuxS-mediated QS in the in vivo pathogenicity of these organisms has not been fully elucidated. In the current study, we constructed a defined deletion mutation in the *luxS* gene of wild-type (WT) rEPEC strain and determined the role of LuxS by in vitro and in vivo assays. The possibility that the QS signals are being supplied by the resident flora is discussed.

2. Materials and methods

2.1. Strains and culture conditions

The bacterial strains and plasmids used in this study are listed in Table 1. The EHEC O157:H7 strain 86-24 and *V. harveyi* BB170 were used as controls (Sperandio et al., 1999). The cloning vector pCR2.1-TOPO (Invitrogen, Carlsbad, CA) was used to clone intermediate PCR products. The positive selection suicide vector plasmid pCVD442 was used for site-directed mutagenesis (Donnenberg and Kaper, 1991). All constructed plasmids were maintained in Top10 One Shot competent cells (Invitrogen) except for pCVD442 and derivatives which were maintained in strains SY327λ*pir* or SM10λ*pir* (Donnenberg and Kaper, 1991). Bacterial strains were grown overnight on Luria–Bertani (LB) agar, or LB broth, or otherwise stated. Appropriate antibiotics were added at the following concentrations: carbenicillin (Car), 50 μg/ml; kanamycin (Kan), 50 μg/ml, nalidixic acid (Nal), 50 μg/ml; tetracycline (Tet), 25 μg/ml.

2.2. Construction of the defined luxS deletion mutation

The defined deletion mutation in the *luxS* gene (the *ygaG* in *E. coli* K-12, GenBank accession #AE000353) was generated by single-overlap extension PCR (SOE-PCR) as previously described (Zhu et al., 2006). Briefly, the 5′ and 3′ regions of the *luxS* gene including the flanking sequences were amplified using two pairs of primers, B621/B657 and B656/B620, respectively (Table 1). Primers B657 and B656 contain 18 nucleotides complementary to each other. The resulting PCR reactions were separated by agarose gel electrophoresis and the amplified bands were excised and