Veterinary Microbiology 165 (2013) 378–383

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

F18ab *Escherichia coli* flagella expression is regulated by acyl-homoserine lactone and contributes to bacterial virulence

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ARTICLE INFO

Article history:
Received 18 February 2013
Received in revised form 14 April 2013
Accepted 19 April 2013

Keywords:
E. coli
AHL
Quorum Sensing
Flagella

ABSTRACT

To investigate the effect of the Quorum Sensing (QS)–I system on the expression of virulence factors in Shiga toxin producing and verotoxin-producing *Escherichia coli* (STEC and VTEC), the *yenI* gene from *Yersinia enterocolitica* was cloned into *E. coli* F18ab 107/86. Recombinant *E. coli* transformed with *yenI* produced acyl-homoserine lactone synthase (AHL), as measured using cross-streaking assays with the reporter biosensor strain *Chromobacterium violaceum* CV026. The AI-1 positive recombinant F18ab *E. coli* exhibited impaired expression of flagella, decreased motility, reduced biofilm formation and AI-2 production, as well as attenuated adherence and invasion on IPEC-J2 cells. This study provides new insights to the crucial function of AI-1 in regulating STEC virulence.

1. Introduction

Escherichia coli are a genetically heterogeneous group of bacteria, a part of the normal microflora of the intestinal tracts of humans and animals. However, intestinal or extraintestinal disease is caused by certain *E. coli* serovars that express virulence genes. Both porcine edema disease (ED) and post-weaning diarrhea (PWD) caused by Shiga toxigenic (STEC) or verotoxigenic *E. coli* (VTEC) result in important morbidity and mortality to pigs (Fairbrother et al., 2005; Nagy and Fekete, 2005), causing significant economic losses to the pig industry. Infections with STEC F18ab$^+$ strains, which produce Shiga toxin 2e (Stx2e), strongly correlate with ED (Imberechts et al., 1996).

Quorum Sensing (QS) is a bacterial communication system that controls the expression of multiple genes in response to bacterial population density (Reading and Sperandio, 2006; Boyen et al., 2009). Small chemical signal molecules called autoinducers (AIs) are produced, released, and detected in the QS process. QS-I was first described in LuxI/LuxR system in the bioluminescent marine bacterium *Vibrio fischeri*. Many bacteria utilize QS to regulate gene expression in response to cell population density to control diverse biological processes, including symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. *E. coli* and *Salmonella* species encode a single LuxR homolog named SdiA, but do not express the LuxI homolog, known as acyl-homoserine lactone (AHL) synthase, which can produce AI-1 (Sabag-Daigle et al., 2012). Acyl homoserine lactone receptor SdiA senses a wide range of AHLs, including, but not limited to N-(3-oxo-octanoyl)-L-homoserine lactone (oxoC8), oxoC6, oxoC10, N-hexanoyl-L-homoserine lactone (C6HSL), N-octanoyl-L-homoserine lactone (C8) (Yao et al., 2006). In the presence of AHLs, a significant proportion of SdiA is expressed in a folded, soluble form in *E. coli*, while it is expressed in nonfunctional, insoluble inclusion bodies in the absence of AI-1

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0378-1135/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.vetmic.2013.04.020

(Yao et al., 2006). SdiA overexpression has been used previously to determine the influence of AI-1 signaling on *E. coli* virulence gene expression (Kanamaru et al., 2000; Yamamoto et al., 2001; Rahmati et al., 2002; Van Houdt et al., 2006).

Flagella are important bacterial virulence factors that provide bacterial motility and, in some cases, contribute to bacterial colonization of host cells, and penetration of the mucosal layer. It has also been proposed that flagella allow enteric bacteria to exploit inflammation to compete with intestinal microbiota *in vivo* (Stecher et al., 2004, 2008; Duan et al., 2012a,b). Flagella expression is often co-regulated with the expression of other virulence factors (Li et al., 2001; Lane and Mobley, 2007a; Lane et al., 2007b; Simms and Mobley, 2008). In this study, we constructed a recombinant F18ab strain containing the *yenI* gene from *Yersinia enterocolitica* (Noel et al., 2010), to express AI-1 in *E. coli* and examined the resultant changes to flagella expression and bacterial virulence.

2. Materials and methods

2.1. Bacterial strains and growth conditions

E. coli 107/86 (wild type, O139:H1:F18ab, Stx2e) (Bertschinger et al., 1990) was routinely cultured in Luria broth (LB) or on Luria agar (LA) plates at 37 °C. *Vibrio harveyi* strains BB170 and the reporter strain *Chromobacterium violaceum* CV026 were kindly provided by Professor Yongjie Liu (Nanjing Agricultural University, China). *Y. enterocolitica* GIM1.266 was purchased from Microbial Culture Collection Center of Guangdong Institute of Microbiology (Guangzhou, China). *V. harveyi* BB170 were used for the bioassay to detect AI-2. *V. harveyi* were cultivated in modified autoinducer bioassay (AB) medium (Han and Lu, 2009a,b). Porcine neonatal jejunal epithelial cell line IPEC-J2 cells were cultured in RPMI 1640-F12 media supplemented with 10% newborn calf serum (NCS) (Gibco) and maintained at 37 °C and 5% CO_2. All other reagents and chemicals were purchased from Sigma (USA).

Construction of F18ab *E. coli* containing the *yenI* gene of *Y. enterocolitica* and negative control strain

The *yenI* gene of *Y. enterocolitica* GIM1 was amplified by PCR using primers *yenI*1 and *yenI*2 (Table 1). The resulting PCR product was digested with *Bam*HI and *Sal*I to yield a DNA fragment spanning the entire *yenI* open reading frame (ORF). The DNA fragment was ligated into the pBR322 plasmid. The recombinant pBR322 plasmid (pBR-*yenI*) was transformed into *E. coli* F18ab strain 107/86. Meanwhile, empty plasmid pBR322 was also transformed into 107/86 to construct the negative control strain. The resulting recombinant strain (F18ab/p*yenI*) produced AHLs, as measured in cross-streaking assays with the biosensor strain CV026, which was differed from wild type and negative control (F18ab/pBR) (Ravn et al., 2001).

2.2. Cross-streaking assays

Cross-streaking assays were performed as described (Dyszel et al., 2010a). Reporter strain CV026 was spread in the middle of an LB plate, while F18ab wild type (WT), F18ab/p*yenI*, AHL positive strain *Y. enterocolitica* GIM1.266, or the negative control strain F18ab/pBR were streaked perpendicular to CV026. After overnight growth, the ability to induce purple color generated by CV026 was assayed.

Table 1
Primers used in this study.

Primer name	Sequences (5′–3′) description
*yenI*1	5′-CGGGGATCC ATGTTAAAACTCTT-3′
*yenI*2	5′-CGCGTCGAC CTATTTAATAATACCAG-3′
gapA-F	5′-CGTTAAAGGCGCTAACTTCG-3′
gapA-R	5′-ACGGTGGTCATCAGACCTTC-3′
fedF-F	5′-CCGTTACTCTTGATTTCTTTGTTG-3′
fedF-R	5′-GGCATTTGGGTAGTGTTTGTCTT-3′
fimH-F	5′-GGCTGCGATGTTTCTGCTC-3′
fimH-R	5′-CCCCAGGTTTTGGCTTTTC-3′
fliC-F	5′-CAGCAAGCGGTGAAGTGAA-3′
fliC-R	5′-AAGCGTAGCCACAGTAGCA-3′
pfs-F	5′-TCACGGCATTTGGTTATGAA-3′
pfs-R	5′-TCGCTTCCATCTCTACAGCA-3′
AIDA-F	5′-CAGTCTACCGCACAAGCAAAAC-3′
AIDA-R	5′-TCAATACACAAAACCCGATACCC-3′

2.3. Bioluminescence detection in AI-2 bioassay

Cell-free culture supernatants were prepared from F18ab WT, F18ab/p*yenI*, F18ab/pBR as described (Han and Lu, 2009a,b). Samples were prepared at different OD_{600} values. AI-2-mediated alterations in bioluminescence were expressed as relative light units of luminescence values measured by Tecan GENios Plus microplate reader in luminescence mode (TECAN GmbH, Austria).

2.4. Bacterial adherence assays

The adherence of STEC 107/86 to IPEC-J2 cells was determined using a quantitative adhesion assay (Scaletsky et al., 1984; Duan et al., 2012a,b). After Triton X-100 treatment, the number of bacteria adherent to IPEC-J2 cells was enumerated.

2.5. Bacterial invasion assays

The cell monolayer was co-incubated with 10^7 CFU bacteria for 2 h, gently washed three times with PBS, and supplemented with 140 mg/mL gentamicin in medium for additional 2 h to kill extracellular bacteria. After Triton X-100 treatment, the number of bacteria invaded to IPEC-J2 cells was enumerated (Duan et al., 2012a,b).

2.6. Motility assays

Bacterial strains were seeded in the center of motility plates. Motility halos were measured in each strain as described previously (Sperandio et al., 2002).

2.7. Crystal violet method for quantification of biofilm formation

Strains were seeded into biofilm-inducing media in either glass test tubes or in 96-well plates as described (Pratt and Kolter, 1998; Li et al., 2008; Duan et al., 2012a,b).