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

Yang Yang [a], Fenghua Yao [a], Mingxu Zhou [a], Jun Zhu [a], Xinjun Zhang [a], Wenbin Bao [b], Shenglong Wu [b], Philip R. Hardwidge [c], Guoqiang Zhu [a,*]

[a] College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
[b] College of Animal Science and Technology, Yangzhou University, Yangzhou University, Yangzhou 225009, China
[c] College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA

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

* Corresponding author. Tel.: +86 514 87972590; fax: +86 514 87311374.
E-mail addresses: yzgqzhu@yzu.edu.cn, yzgqzhu@hotmail.com (G. Zhu).

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).

The OD_{600} values of each well were read to measure the amount of biofilm production using a crystal violet staining method. Each strain was tested by six repetitions and the experiment was repeated three times.

2.8. Negative staining and TEM

Flagellar morphology was visualized by negative staining followed by Transmission electron microscope (TEM) (Duan et al., 2012a,b). After being negatively stained with 1% phosphotungstic acid (pH 7.4) for 2 min on carbon-formvar copper grids, flagella were examined with a Philips CM-100 TEM at a voltage of 60 kV.

2.9. RNA isolation and real-time quantitative polymerase chain reaction (qRT-PCR)

Total RNA from each strain was extracted using TRIzol (Han and Lu, 2009a,b; Duan et al., 2013). Primers were designed to amplify unique sequence regions of the *fimH*, *fedF*, *fliC*, *pfs*, and *AIDA-1* genes. All data were normalized to the endogenous reference gene *gapA*. Assays were performed using an ABI 7500 real-time PCR system (Applied Biosystems, USA). Data were analyzed using the $2^{-\Delta\Delta CT}$ method.

3. Statistical analysis

All statistical analyses were performed using SPSS 15.0 software (SPSS Inc., USA). Differences in data were analyzed with *t*-tests. In all cases, significance was defined as $P < 0.05$.

4. Results

Expressing *yenI* in F18ab *E. coli* alters the expression of flagella, bacterial motility, and biofilm formation.

Expression of pBR-*yenI* in F18ab *E. coli* conferred the production of AI-1, as measured by the induction of purple color by the reporter strain CV026. *Y. enterocolitica* GIM1.266 was used as a positive control, whereas no AHL production was detected in either F18ab WT or in the negative control F18ab/pBR (Fig. 1). Whereas both WT F18ab *E. coli* and F18ab/pBR produced flagella, as assessed by TEM, flagella were not observed in the recombinant F18ab/*pyenI* strain (Fig. 2), suggesting that expressing AI-1 repressed the production of flagella. Accordingly, the swimming motility halo of F18ab/p*yenI* was smaller (~30%) than that of F18ab WT and F18ab/pBR (Fig. 3).

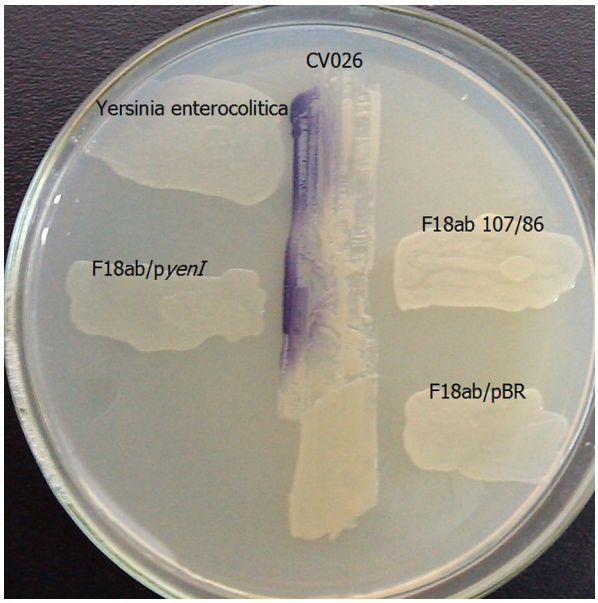

Fig. 1. AI-1 activity was tested using the biosensor strain *Chromobacterium violaceum* CV026. AI-1 activity was tested in cross-streaking assays. F18ab WT, F18ab/p*yenI*, *Yersinia enterocolitica* GIM1.266, and F18ab/pBR were streaked across the biosensor strain, respectively.

4.1. AHL production influences F18ab E. coli adherence and invasion to IPEC-J2 cells.

The adherence of F18ab/p*yenI* to IPEC-J2 cells was significantly reduced (66%) as compared with the adherence of the WT strain and F18ab/pBR (Fig. 4A). Similarly, the invasion of F18ab/p*yenI* was also reduced (65%) as compared with WT and negative control strain (Fig. 4B). Results from qRT-PCR assays showed that *yenI* expression caused a 6-fold reduction in the expression of flagellin

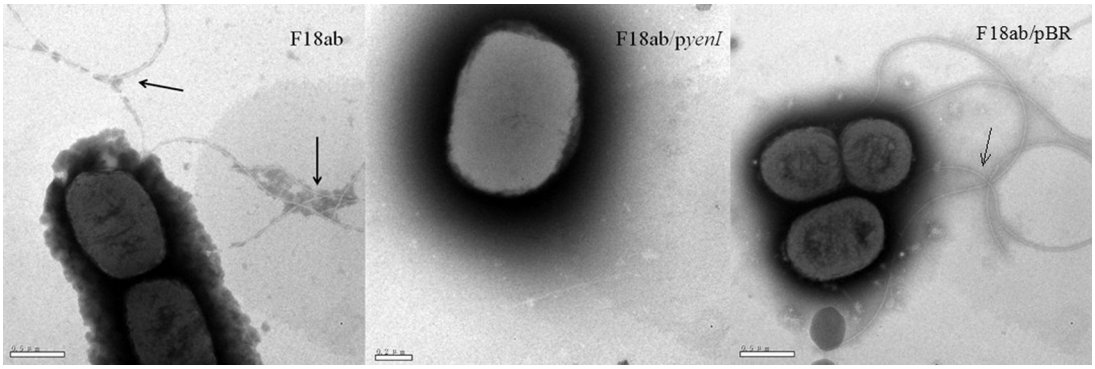

Fig. 2. Flagella expression was observed and assessed by TEM. Arrows indicate flagella.

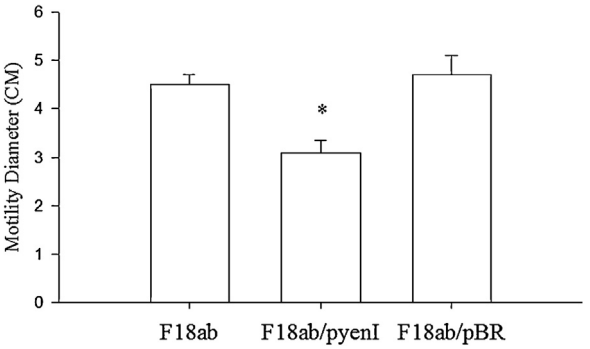

Fig. 3. Swim motility assays. The motility diameter was measured after 12 h growth on 0.3% swim agar plates. Statistical significance was determined by *t* test based on comparison with 107/86 strain (*$P < 0.05$).

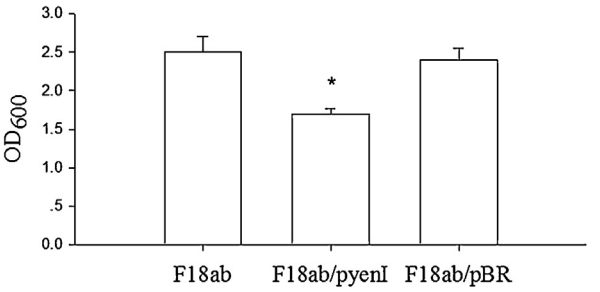

Fig. 5. Effect of *yenI* on biofilm formation. Qualitative analyses of biofilm formation were exerted. Each strain was seeded into biofilm inducing medium, and the results by crystal violet method were observed in OD_{600}. Statistical significance was determined by *t* test based on comparison with 107/86 strain (*$P < 0.05$).

(*fliC*), but 3-fold and 2-fold increases in the expression of type I fimbriae (*fimH*) and AIDA-1 adhesin, respectively (Fig. 7). No significant change in F18 fimbriae (*fedF*) expression was detected (data not shown). These data suggest that AHL expression in F18ab *E. coli* negatively impacts adherence and invasion.

4.2. Influence of AHLs in F18ab E. coli biofilm formation

Glass tube surface biofilm formation by various bacteria was also tested as a purple ring of crystal violet-stained cells that formed at the interface between the air and the liquid culture medium. F18ab/p*yenI* was much more reduced (data not shown). When the biofilm in 96-well microtiter plate was quantified by optical absorbance at OD_{600} nm after solubilizing with ethanol, F18ab/p*yenI* exhibited approximately 67% of the WT and F18ab/pBR

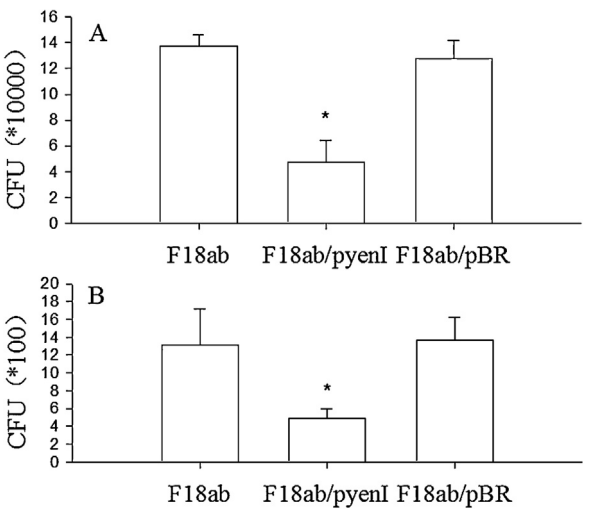

Fig. 4. Effect of *yenI* on adherence and invasion of 107/86 to IPEC-J2 cells. (A) Quantification of adherence of F18ab WT, F18ab/p*yenI* and F18ab/pBR to IPEC-J2 cells. B. Quantification of invasion of F18ab WT, F18ab/p*yenI*, F18ab/pBR into IPEC-J2 cells. *Y*-axis indicates average CFU values recovered from each well of 96-well plate. Statistical significance was determined by t test based on comparison with 107/86 strain (*$P < 0.05$).

absorbance (Fig. 5). Thus, AHLs produced by *yenI* seems to play an important role in biofilm formation of F18ab *E. coli*.

4.3. Influence of AI-1 on AI-2 expression in F18ab E. coli

AI-2 activity was impaired from lag phase to the late log phase in F18ab/p*yenI*. This result indicates the possible influence of AI-1 on AI-2 production (Fig. 6). This data was consistent with the reduction mRNA level of *pfs*, which contributes to AI-2 synthesis in *E. coli*.

5. Discussion

To examine the effect of AI-1 on flagella expression in *E. coli*, we complemented F18ab with the *Y. enterocolitica yenI* gene, to produce AHLs endogenously. This strategy avoids the potential experimental complications of SdiA over-expression (Dyszel et al., 2010b) and/or reliance on exogenous AHLs (Yao et al., 2006). The AI-1 positive STEC strain, F18ab/p*yenI*, displayed reduced flagella expression and reduced motility.

Providing motility is not the only role of flagella. Expression of flagellin has been shown to be required for maximal bacterial adherence, colonization, and subsequent invasion (Smith et al., 2003; Duan et al., 2012a,b). In

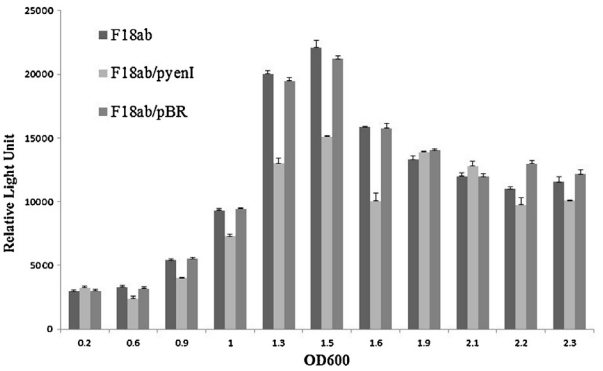

Fig. 6. AI-2 activity was measured using the *V. harveyi* bioluminescence assay. Samples were extracted at different values of OD_{600} in each strain. AI-2 activity is expressed as relative light units measured.

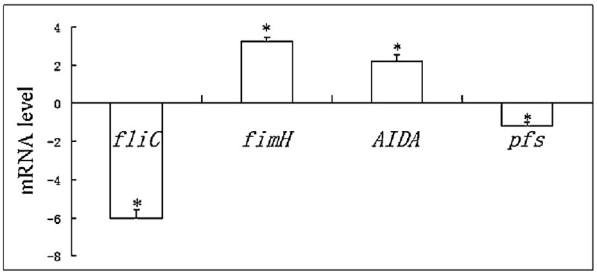

Fig. 7. Transcriptional level of various genes in F18ab/p*yenI*. All data were normalized to the endogenous reference gene *gapA*. Values indicate changed folds of expression level of genes in F18ab/p*yenI*, compared with F18ab WT and F18ab/pBR. Means marked with * are significantly different at *P* values of 0.05.

our previous study, IPEC-J2 is a suitable cell model for flagella-adherence experiment *in vitro* (Duan et al., 2012a,b, 2013). The assays showed the adherence and invasion ability of F18ab *E. coli* were damaged drastically, which proved the impaired expression of flagella. In our previous study (Duan et al., 2013), expression of *fimH* (subunit of type I fimbriae) was up-regulated in the *fliC* mutant strain by 2-fold increase, which was consistent with the observation in this study, as 3.25-fold increase was observed. Expression of non-fimbriae adhesin AIDA was also increased by 2.2-fold. This complicated relationship between different adhesins was consistent with previous studies that have shown that the loss of one adhesin generally leads to up-regulation of the expression of another adhesin type. Overexpression of type I fimbriae, P fimbriae and proteuslike (MR/P) fimbriae could repress the expression of flagella (Li et al., 2001; Lane and Mobley, 2007a; Lane et al., 2007b; Simms and Mobley, 2008). However, the precise mechanism that regulates the expression balance among flagella, type I fimbriae or AIDA is still unclear.

Biofilms are communities of microorganisms attached to a solid surface through extracellular polymeric substances. It has been shown that flagella contribute not only to early attachment but also to biofilm development and maturation. Flagella might act in the early steps of biofilm formation as both surface adhesins and providers of motility, which may be indirectly involved in pathogenicity by participating in biofilm formation (Duan et al., 2012a,b). A 33% reduction of biofilm formation was observed in F18ab/p*yenI*. In many cases, biofilm formation is related to many essential virulence factors contributing to colonization, immune escape, and antibiotic resistance (Costerton et al., 1999; Hall-Stoodley et al., 2004; Li et al., 2008). We assumed that through flagella, many properties of pathogenesis have been adjusted under the regulation of AI-1.

Gram-positive and Gram-negative bacteria share the QS-II system, which manipulates the expression of multiple genes through AI-2. The AI-2 synthase LuxS was identified widely among bacteria. LuxS, with the Pfs protein (5′-methyl thioadenosine/S-adenosylhomocysteine nucleosidase) is involved in AI-2 biosynthesis derived from S-adenosylmethionine (SAM) (Sperandio et al., 2001). AI-2 can be measured quantitatively by inducing luminescence in the AI-2 reporter strain *V. harveyi* BB170. In many pathogenic bacteria, AI-2 plays an important role in regulating bacterial virulence strategies, including type III secretion systems, pathogenicity, and biofilm formation (Walters and Sperandio, 2006). In our previous study, a slight reduction of AI-2 production was tested in *fliC* deletion K88 enterotoxigenic *E. coli* (ETEC). Because LuxS and Pfs were known as important functional enzymes in synthesis of AI-2, mRNA levels in K88 wild type and *fliC* deletion strain were determined by qPCR. A 20–25% decrease of *luxS* and *pfs* mRNA were tested in mutant strain, respectively (unpublished data). Therefore, we decided to study whether AI-2 production was also related with suppressed flagella in F18ab *E. coli* in existence of AI-1. Although expression of *luxS* was not decreased significantly (data not shown), an 18% reduction was detected in *pfs* of F18ab strain. Previous study showed that the level of transcription of *pfs* is highly correlated with AI-2 production in *Streptococcus suis* Serotype 2 (SS2), but that transcription of *luxS* does not correlate with the level of AI-2, although *luxS* is required for AI-2 synthesis (Han and Lu, 2009a,b). We assumed in *E. coli*, *pfs* is also the rate-limiting enzyme. Although the mechanism is not determined clearly, this result provides a new insight into influence of flagella on AI-2, as AI-2 was only known as an important factor which regulates flagella expression in *E. coli*, and the complicated interaction between QS-I and QS-II remains to be further detected.

In conclusion, this study demonstrated that AI-1 affects the expression of flagella, one of the important F18ab *E. coli* virulence factors (Han and Lu, 2009a,b). The activity of the AI-1 in STEC may partly account for the attenuated virulence from the suppressed expression of *fliC*. This study provides insight into virulence genes regulated by AI-1. Moreover, disruption of AI-1 mediated Quorum Sensing has been considered as a new anti-infective strategy to control infectivity and pathogenicity of STEC.

Conflict of interest

The authors or their institution do not have any relationships that may influence or bias the results and data presented in this manuscript.

Acknowledgments

This study was supported by grants from the Chinese National Science Foundation Grants (Nos. 31072136, 30771603 and 31270171), the Jiangsu High Education Key Basic Science Foundation (08KJA230002), and the Genetically Modified Organisms Technology Major Project of China (2009ZX08006-004B), a project founded by the Priority Academic Program of Development Jiangsu High Education Institution, program granted for scientific innovation research of college graduate in Jangsu province (CXLX12-0937).

References

Bertschinger, H.U., Bachmann, M., Mettler, C., Pospischil, A., Schraner, E.M., Stamm, M., Sydler, T., Wild, P., 1990. Adhesive fimbriae produced in vivo by *Escherichia coli* O139:K12(B):H1 associated with enterotoxaemia in pigs. Vet. Microbiol. 25 (2–3) 267–281.

Boyen, F., Eeckhaut, V., Van Immerseel, F., Pasmans, F., Ducatelle, R., Haesebrouck, F., 2009. Quorum sensing in veterinary pathogens: mechanisms, clinical importance and future perspectives. Vet. Microbiol. 135 (3–4) 187–195.

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science 284 (5418) 1318–1322.

Duan, Q., Zhou, M., Zhu, L., Zhu, G., 2012a. Flagella and bacterial pathogenicity. J. Basic Microb. 52, 1–8.

Duan, Q., Zhou, M., Zhu, X., Bao, W., Wu, S., Ruan, X., Zhang, W., Yang, Y., Zhu, J., Zhu, G., 2012b. The flagella of F18ab *Escherichia coli* is a virulence factor that contributes to infection in a IPEC-J2 cell model in vitro. Vet. Microbiol. 160 (1–2) 132–140.

Duan, Q., Zhou, M., Zhu, X., Yang, Y., Zhu, J., Bao, W., Wu, S., Ruan, X., Zhang, W., Zhu, G., 2013. Flagella from F18+ *Escherichia coli* play a role in adhesion to pig epithelial cell lines. Microb. Pathog. 55, 32–38.

Dyszel, J.L., Smith, J.N., Lucas, D.E., Soares, J.A., Swearingen, M.C., Vross, M.A., Young, G.M., Ahmer, B.M., 2010a. *Salmonella enterica* serovar Typhimurium can detect acyl homoserine lactone production by *Yersinia enterocolitica* in mice. J. Bacteriol. 192 (1) 29–37.

Dyszel, J.L., Soares, J.A., Swearingen, M.C., Lindsay, A., Smith, J.N., Ahmer, B.M., 2010b. *E. coli* K-12 and EHEC genes regulated by SdiA. PLoS ONE 5 (1) e8946.

Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6 (1) 17–39.

Hall-Stoodley, L., Costerton, J.W., Stoodley, P., 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nat. Rev. Microbiol. 2 (2) 95–108.

Han, X.G., Lu, C.P., 2009a. Detection of autoinducer-2 and analysis of the profile of luxS and pfs transcription in *Streptococcus suis* serotype 2. Curr. Microbiol. 58 (2) 146–152.

Han, X.G., Lu, C.P., 2009b. In vitro biosynthesis of autoinducer 2 of *Steptococcus suis* Serotype 2 using recombinant LuxS and Pfs. Enzyme Microb. Technol. 44 (1) 40–45.

Imberechts, H., Wild, P., Charlier, G., De Greve, H., Lintermans, P., Pohl, P., 1996. Characterization of F18 fimbrial genes *fedE* and *fedF* involved in adhesion and length of enterotoxemic *Escherichia coli* strain 107/86. Microb. Pathog. 21 (3) 183–192.

Kanamaru, K., Kanamaru, K., Tatsuno, I., Tobe, T., Sasakawa, C., 2000. SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. 38 (4) 805–816.

Lane, M.C., Mobley, H.L., 2007a. Role of P-fimbrial-mediated adherence in pyelonephritis and persistence of uropathogenic *Escherichia coli* (UPEC) in the mammalian kidney. Kidney Int. 72 (1) 19–25.

Lane, M.C., Simms, A.N., Mobley, H.L., 2007b. complex interplay between type 1 fimbrial expression and flagellum-mediated motility of uropathogenic *Escherichia coli*. J. Bacteriol. 189 (15) 5523–5533.

Li, L., Zhou, R., Li, T., Kang, M., Wan, Y., Xu, Z., Chen, H., 2008. Enhanced biofilm formation and reduced virulence of *Actinobacillus pleuropneumoniae luxS* mutant. Microb. Pathog. 45 (3) 192–200.

Li, X., Rasko, D.A., Lockatell, C.V., Johnson, D.E., Mobley, H.L., 2001. Repression of bacterial motility by a novel fimbrial gene product. EMBO J. 20 (17) 4854–4862.

Nagy, B., Fekete, P.Z., 2005. Enterotoxigenic *Escherichia coli* in veterinary medicine. Int. J. Med. Microbiol. 295 (6-7) 443–454.

Noel, J.T., Joy, J., Smith, J.N., Fatica, M., Schneider, K.R., Ahmer, B.M., Teplitski, M., 2010. Salmonella SdiA recognizes N-acyl homoserine lactone signals from *Pectobacterium carotovorum* in vitro, but not in a bacterial soft rot. Mol. Plant Microbe Interact. 23 (3) 273–282.

Pratt, L.A., Kolter, R., 1998. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol. Microbiol. 30 (2) 285–293.

Rahmati, S., Yang, S., Davidson, A.L., Zechiedrich, E.L., 2002. Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. Mol. Microbiol. 43 (3) 677–685.

Ravn, L., Christensen, A.B., Molin, S., Givskov, M., Gram, L., 2001. Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics. J. Microbiol. Methods 44 (3) 239–251.

Reading, N.C., Sperandio, V., 2006. Quorum sensing: the many languages of bacteria. FEMS Microbiol. Lett. 254 (1) 1–11.

Sabag-Daigle, A., Soares, J.A., Smith, J.N., Elmasry, M.E., Ahmer, B.M., 2012. The acyl homoserine lactone receptor, SdiA, of *Escherichia coli* and *Salmonella enterica serovar Typhimurium* does not respond to indole. Appl. Environ. Microbiol. 78 (15) 5424–5431.

Scaletsky, I.C., Silva, M.L., Trabulsi, L.R., 1984. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect. Immun. 45 (2) 534–536.

Simms, A.N., Mobley, H.L., 2008. PapX, a P fimbrial operon-encoded inhibitor of motility in uropathogenic *Escherichia coli*. Infect. Immun. 76 (11) 4833–4841.

Smith, K.D., Andersen-Nissen, E., Hayashi, F., Strobe, K., Bergman, M.A., Barrett, S.L., Cookson, B.T., Aderem, A., 2003. Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat. Immunol. 4 (12) 1247–1253.

Sperandio, V., Torres, A.G., Kaper, J.B., 2002. Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. Mol. Microbiol. 43 (3) 809–821.

Sperandio, V., Torres, A.G., Giron, J.A., Kaper, J.B., 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. J. Bacteriol. 183 (17) 5187–5197.

Stecher, B., Barthel, M., Schlumberger, M.C., Haberli, L., Rabsch, W., Kremer, M., Hardt, W.D., 2008. Motility allows *S. Typhimurium* to benefit from the mucosal defence. Cell. Microbiol. 10 (5) 1166–1180.

Stecher, B., Hapfelmeier, S., Muller, C., Kremer, M., Stallmach, T., Hardt, W.D., 2004. Flagella and chemotaxis are required for efficient induction of *Salmonella enterica serovar Typhimurium* colitis in streptomycin-pretreated mice. Infect. Immun. 72 (7) 4138–4150.

Van Houdt, R., Aertsen, A., Moons, P., Vanoirbeek, K., Michiels, C.W., 2006. N-acyl-L-homoserine lactone signal interception by *Escherichia coli*. FEMS Microbiol. Lett. 256 (1) 83–89.

Walters, M., Sperandio, V., 2006. Quorum sensing in *Escherichia coli* and *Salmonella*. Int. J. Med. Microbiol. 296 (2-3) 125–131.

Yamamoto, K., Yata, K., Fujita, N., Ishihama, A., 2001. Novel mode of transcription regulation by SdiA, an *Escherichia coli* homologue of the quorum-sensing regulator. Mol. Microbiol. 41 (5) 1187–1198.

Yao, Y., Martinez-Yamout, M.A., Dickerson, T.J., Brogan, A.P., Wright, P.E., Dyson, H.J., 2006. Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. J. Mol. Biol. 355 (2) 262–273.