CrossMark

ORIGINAL ARTICLE

Type I fimbriae mediate in vitro adherence of porcine F18ac+ enterotoxigenic *Escherichia coli* (ETEC)

Qiangde Duan[1] · Rahul Nandre[2] · Mingxu Zhou[3] · Guoqiang Zhu[3]

Abstract Type I fimbriae commonly expressed by *Escherichia coli* mediate initial attachment of bacteria to host epithelial cells. However, the role of type I fimbriae in the adherence of porcine enterotoxigenic *E. coli* (ETEC) to host receptors is unclear. In this study, we examined the role of type I fimbriae in the adherence and biofilm formation of F18ac+ ETEC by constructing mutant strains with deletion of type I fimbrial major subunit (*fimA*) or minor subunit (*fimH*). The data indicated that the isogenic Δ*fimA* and Δ*fimH* mutants showed significantly lower adherence to porcine epithelial IPEC-1 and IPEC-J2 cells as compared to the F18ac+ ETEC parent strain. In addition, the adherence of F18ac+ ETEC to both cell lines was blocked by the presence of 0.5% D-mannose in the cell culture medium. In addition, both mutant strains impaired their ability to form biofilm in vitro. Interestingly, the deletion of *fimA* or *fimH* genes resulted in remarkable up-regulation of the expression of adhesin involved in diffuse adherence (AIDA-I). These results indicated that type I fimbriae may be required for efficient adherence of F18ac+ ETEC to pig epithelial cells and, perhaps, biofilm formation.

✉ Qiangde Duan
254666842@qq.com; dqd8358@163.com

✉ Guoqiang Zhu
yzgqzhu@yzu.edu.cn

1 Weinan Vocational and Technical College, Weinan 714000, China

2 College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA

3 College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China

Introduction

Post-weaning diarrhea (PWD) causes severe economic losses to swine production due to high morbidity and mortality (Fairbrother et al. 2005; Rausch et al. 2017). Enterotoxigenic *Escherichia coli* (ETEC) strains expressing F18ac fimbriae (F18ac+ ETEC) or K88 fimbriae (K88+ ETEC) are the most common bacterial pathogens causing PWD (de la Fé Rodríguez et al. 2011; Frydendahl 2002; Zhang et al. 2007). Adhesins and enterotoxins [heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST)] of ETEC are known as virulence determinants in PWD (Fairbrother et al. 2005; Zhang et al. 2007). Adhesins expressed by F18ac+ ETEC initially mediate adherence to pig small intestinal epithelial cells. Adhered bacteria then produce and release enterotoxins into the gut lumen, leading to fluid homeostasis and PWD. Thus, it is believed that initial adherence mediated by classes of adhesins is critical for F18ac+ ETEC infection. Adhesins involved in the F18ac+ ETEC adherence process include F18ac fimbriae and flagella (Frydendahl 2002; Duan et al. 2013). F18ac fimbriae mediate F18ac+ ETEC adherence to specific host cell receptors by their adhesive subunit (*fedF*). Flagella are suggested to contribute to F18ac+ ETEC adherence in two ways: (i) promoting bacteria attachment to host tissue by their motility property; (ii) directly increasing the adherence mechanism as adhesins. Adhesins involved in diffuse adherence (AIDA-I) are expressed by F18ac+ ETEC. Additionally, these contribute to colonization and biofilm formation for porcine diarrheagenic *E. coli* strain PD20 (Ravi et al. 2007).

Type I fimbriae are the most commonly expressed pili on the surface of *E. coli*. They are encoded by the *fim* gene cluster

that includes nine genes (*fimA*–*fimH*) and are required for fimbrial biosynthesis (Orndorff and Falkow 1984; Klemm and Christiansen 1987). The major structural fimbrial subunit is encoded by the *fimA* gene, whereas the minor and adhesive subunit, which is located at the tip of the fimbriae, is encoded by the *fimH* gene (Klemm 1984; Krogfelt et al. 1990). Type I fimbriae mediate bacterial adherence by binding its *fimA* adhesin subunit to mannose-containing glycoprotein receptors on host cells. It is also known that type I fimbriae are important adhesins for many pathogens. In uropathogenic *E. coli* (UPEC) causing urinary tract infections (UTIs), type I fimbriae are implicated as virulence factors for UPEC bacterial initial adherence and subsequent invasion to urinary epithelial cells and biofilm formation (Anderson et al. 2003; Wiles et al. 2008; Bien et al. 2012). Type I fimbriae mediate adhesion and invasion to human brain microvascular endothelial cells (HBMEC) to develop neonatal meningitis (Murphy et al. 2013). Additionally, type I fimbriae of *E. coli* strain LF82 mediate adherence to host cells in Crohn's disease (Boudeau et al. 2001). Also, the type I fimbriae are commonly expressed in F18ac+ ETEC strains. However, their role(s) in adherence to pig epithelial cells remains poorly understood.

In this study, we constructed mutants with deletion of the major subunit (F18acΔ*fimA*) or the minor subunit (F18acΔ*fimH*) and examined the ability of the mutant strains to adhere to piglet epithelial IPEC-1 and IPEC-J2 cell lines. In addition, we explored the cross-regulation of type I fimbriae and other adhesins of F18ac+ ETEC.

Materials and methods

Bacterial strains, plasmids, and cell lines

Bacterial strains and plasmids used in this study are listed in Table 1. Antibiotic-resistant strains were cultured in LB containing 100 μg/mL of ampicillin (Amp) or 34 μg/ml of chloramphenicol (Cm). For determining bacterial growth rates, wild-type and mutants strains were grown in LB broth, and complemented strains in LB broth containing 100 μg/mL of Amp, for 6 h at 37 °C in a shaking incubator at 220 rpm. Afterwards, the optical density was measured hourly for each cultured strain at 600 nm (OD_{600}).

Newborn porcine small intestinal epithelial cell lines IPEC-J2 and IPEC-1 were cultured in Dulbecco's minimal Eagle medium (DMEM) (Gibco) supplemented with heat-inactivated fetal bovine serum (FBS; Gibco) in 5% CO_2 at 37 °C.

Mutant strains and complementary strains construction

The F18ac+ ETEC *fimA* (GenBank: CP019558.1) and *fimH* (GenBank: JF289169.1) isogenic gene mutants were constructed using the phage λ-red-mediated recombination system as previously described (Datsenko and Wanner 2000). Briefly, the Cm resistance-encoding gene sequence was amplified from the chloramphenicol cassette of plasmid pKD3 in polymerase chain reaction (PCR) amplification with ΔfimA-F and ΔfimA-R primers (Table 2). Amplified PCR products were purified from agarose gels and transferred into F18ac+ ETEC-competent cells containing pKD46 plasmid. Positive colonies were selected on LB agar plates containing Amp (100 μg/mL) and Cm (34 μg/mL). Allelic replacement of *fimA* by the Cm cassette was verified by PCR screening using *fimA*-specific primers (pBR-fimA-F, pBR-fimA-R; Table 2). Finally, Flp recombinase-expressing vector pCP20 was introduced to delete the Cm cassette. Similarly, the Δ*fimH* mutant was generated using ΔfimH-F and ΔfimH-R primers (Table 2). Deletion of *fimA* or *fimH* in the mutant strains was confirmed by PCR screening, DNA sequencing, and hemagglutination assays.

Table 1 Bacterial strains and plasmids used in this study

Strains/plasmids	Characteristics	Reference source
2134P	Wild-type, O157:H19:F18ac, 4P−, STa+, STb+	Casey et al. (1992)
F18acΔ*fimA*	*fimA* deletion mutant of 2134P	In this study
F18acΔ*fimH*	*fimH* deletion mutant of 2134P	In this study
F18acΔ*fimA*/p*fimA*	F18acΔ*fimA* carrying pBR-fimA	In this study
F18acΔ*fimH*/p*fimH*	F18acΔ*fimA* carrying pBR-fimH	In this study
Plasmids		
pKD3	Cm^r; Cm cassette template	Datsenko and Wanner (2000)
pKD46	Amp^r, λ-red recombinase expression	Datsenko and Wanner (2000)
pCP20	Amp^r, Cm^r; Flp recombinase expression	Datsenko and Wanner (2000)
pBR322	Expression vector, Amp^r	Takara
pBR-fimA	pBR322 carrying intact ORF of *fimA*	In this study
pBR-fimH	pBR322 carrying intact ORF of *fimH*	In this study

Table 2 Oligonucleotides used in this study

ΔfimA-F	TGGGACCGTTCACTTTAAAGGGGAAGTTGTTA ACGC
	CGCTTGCGCAGTTTGTGTAGGCTGGAGCTGCT TCG
ΔfimA-R	GGTTGCAAAATAACGCGCCTGGAACGGAATGG TGTT
	GGTTCCGTTATTCATATG AATATCCTCCTTAG
ΔfimH-F	ACGTGCGTAATTTGCCGTTAATCCCAGACTTA CCGCC
	GAAGTCCCTACCATAT GAATATCCTCCTTAG
ΔfimH-R	ACGTGCGTAATTTGCCGTTAATCCCAGACTTA CCGCC
	GAAGTCCCTACCATATG AATATCCTCCTTAG
pBR-fimA-F	CGCGGATCCATGAAAATTAAAACTCTGGCA
pBR-fimA- R	CAGCGTCGACTTATTGATACTGAACCTTGA
pBR-fimH-F	CGCGGATCCATGAAACGAGTTATTACC CTGTT
pBR-fimH-R	CAGCGTCGACTTATTGATAAACAAAAG TCACGCC
gapA-F	CGTTAAAGGCGCTAACTTCG
gapA-R	ACGGTGGTCATCAGACCTTC
fliC-F	ACTCAGAAAACCTGATGGTGAAACT
fliC-R	CCCCACCTCTCCCTAACACA
fedF-F	CCGTTACTCTTGATTTCTTTGTTG
fedF-R	GGCATTTGGGTAGTGTTTGTCTT
AIDA-F	CAGTCTACCGCACAAGCAAAC
AIDA-R	TCAATACACAAAACCCGATACCC

The full-length *fimA* and *fimH* genes were PCR-amplified from F18ac+ ETEC genomic DNA using primers pBR-fimA-F/pBR-fimA-R and pBR-fimH-F/pBR-fimH-R, respectively (Table 2). Plasmids pBR322/fimA or pBR322/fimH were introduced into F18acΔ*fimA* or F18acΔ*fimH* to generate complementary strains. Restoration of type I fimbriae expression in the complementary strains was verified in yeast cell agglutination and hemagglutination assays.

Yeast cell agglutination assay and mannose-sensitive hemagglutination (MSHA) test

The yeast cell agglutination assay was performed as previously described (Müller et al. 2009). Briefly, bacteria that were subcultured four times by serial two-fold dilution in LB broth at 37 °C under static conditions and *Pichia pastoris* GS115 were washed and resuspended to $OD_{600} = 0.5$ using phosphate buffered saline (PBS, pH7.4) with or without 0.5% D-mannose. Suspensions were mixed at a 1:1 (v/v) ratio. The slide was gently rotated for 5 min at room temperature (RT), while monitoring for visible HA was conducted. In the MSHA assay, 25 μL of 5% pig erythrocyte suspension with or without D-mannose were mixed with an equal volume of a bacterial suspension on a glass slide. The agglutination was evaluated after being gently rotated for 5 min at RT.

Adherence assays and adherence inhibition assays

Bacterial adherence assays were performed as previously described (Jouve et al. 1997). Briefly, in each well of a 48-well tissue culture plate (Corning), 1×10^5 IPEC-1or IPEC-J2 cells were challenged with *E. coli* at 2×10^6 colony-forming units (CFUs) for 1 h at 37 °C. Cells were washed three times with PBS to remove non-adherent bacteria, then lysed with 0.5% Triton X-100. Cell lysates were serially diluted with PBS and plated on LB agar plates overnight at 37 °C.

The adherence inhibition assay was performed by coincubation of the F18ac+ ETEC parent strains and cells in the presence of 0.5% D-mannose. The number of adherent bacteria was determined as described above.

Quantification of biofilm formation

Biofilm formation in a 96-well microtiter plates assay was carried out as previously described (Duan et al. 2013). Briefly, overnight-grown bacterial cultures were adjusted to $OD_{600} = 1.0$ using fresh biofilm-inducing medium (Hossain and Tsuyumu 2006). 150-μL culture suspensions were added to each well of the 96-well microtiter plates and incubated for 24 h at 30 °C statically. Non-adherent bacteria in the wells were removed by washing with ddH$_2$O. Biofilm was stained with 200 μL of 2% crystal violet (CV) at RT for 15 min and solubilized by 95% ethanol for 30 min at RT. OD_{600} values were measured by a spectrophotometer (BioTek, USA).

RNA isolation and real-time quantitative polymerase chain reaction (RT-PCR)

Total RNA from bacterial samples was extracted using a TRIzol as previously described (Duan et al. 2013). RNA quality and yield were verified with agarose gel analysis. The PrimeScript® RT Reagent Kit with gDNA Eraser (Takara) was used to synthesize the cDNA according to the manufacturer's protocol. Primers were designed to amplify *fliC*, *AIDA-I*, and *fedF* genes to target regions of unique sequences within each gene (Table 2). PCRs were performed in triplicate with the ABI7500 instrument. All data were normalized with the endogenous reference gene *gapA* and analyzed by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Each experiment was performed in triplicate and repeated at least three times to ensure reproducibility. Data were analyzed using Student's *t*-test for independent samples. Differences were considered significant if $p \leq 0.05$.

Results

Characterization of ΔfimA and ΔfimH mutants and complementary strains

Construction of F18ac+ ETEC mutants and the complemented strains (F18acΔfimA/pfimA and F18acΔfimH/pfimH) were verified by DNA sequencing and agglutination reactions. Unlike the parent strain, the F18acΔfimA and F18acΔfimH mutant strains lost their ability to agglutinate with yeast cells and pig erythrocytes. In contrast, the complementary strains restored their ability to agglutinate with yeast cells and pig erythrocytes in a mannose-sensitive manner like the parent strains. All strains had similar growth rate after culturing in LB broth in a shaking incubator for 6 h at 37 °C (complemented strains in LB broth with Amp) (Fig. 1).

Type I fimbriae mediate F18ac+ ETEC adherence to IPEC-1 and IPEC-J2 cells

F18ac+ ETEC strains bind to IPEC-1 and IPEC-J2 porcine cell lines that are derived from the small intestine of newborn piglets (Duan et al. 2013). It was found that both F18acΔfimA and F18acΔfimH mutants showed reduced adherence ability to either the IPEC-1 or IPEC-J2 cell lines. Cell viable counts revealed that the F18acΔfimA mutant exhibited about 38% reduction in adherence compared to that of the parent strain, and the F18acΔfimH mutant showed about 43% reduction of adherence (Fig. 2a). In a similar way, F18acΔfimA and F18acΔfimH mutants showed 62% and 64% adherence to IPEC-J2 cells (Fig. 2b). In contrast, F18acΔfimA/pfimA and F18acΔfimH/pfimH complemented strains showed similar adherence to both cell lines as the parent strains.

The adherence inhibition assay showed that the adherence of wild-type F18ac+ ETEC strains to IPEC-1 and IPEC-J2 cells was blocked 51% and 73%, respectively, by 0.5%
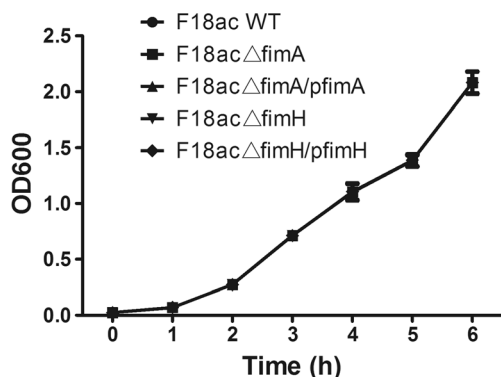
Fig. 1 Bacterial growth curves of F18+ enterotoxigenic *Escherichia coli* (ETEC) wild-type (WT), mutant strains, and complementary strains. WT F18ac+ ETEC, F18acΔfimA, F18acΔfimA/pfimA, F18acΔfimH, and F18acΔfimH/pfimH were grown in LB broth at 37 °C for 6 h. The optical density at 600 nm (OD$_{600}$) was measured hourly. The data presented are the means of four independent measurements
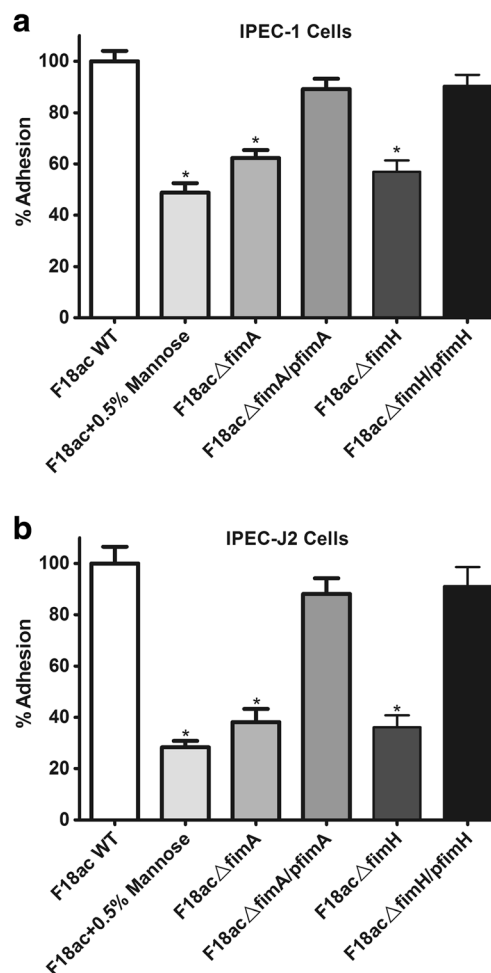
Fig. 2 Adherence of WT F18ac+ ETEC, isogenic mutants, and complementary strains to IPEC-1 and IPEC-J2 cells. **a** Adherence to IPEC-1 cells by WT F18ac+ ETEC, F18acΔfimA, F18acΔfimA/pfimA, F18acΔfimH, and F18acΔfimH/pfimH strains. **b** Adherence to IPEC-J2 cells by WT F18ac+ ETEC, F18acΔfimA, F18acΔfimA/pfimA, F18acΔfimH, and F18acΔfimH/pfimH strains. The adherence index of WT strains was set as 100%. Each assay was conducted in triplicate and independently repeated at least three times. *Indicates statistically significant differences to the F18ac+ ETEC WT strain ($p < 0.05$)

D-mannose compared with wild-type strains. The magnitude inhibition of 8% D-mannose was not significantly different compared to that from 0.5% D-mannose (data not shown). The results indicated that type I fimbriae were involved in F18ac+ ETEC adherence to both IPEC-1 and IPEC-J2 cells in vitro.

Type I fimbriae enhance F18ac+ ETEC biofilm formation

F18ac+ ETEC strains formed biofilm in the 96-well microplates in vitro. F18acΔfimA and F18acΔfimH mutants exhibited about 41% and 69% absorbance, respectively, compared to the parent strain absorbance (Fig. 3, $p < 0.01$). In contrast, the biofilm formation ability of both complemented strains was restored.
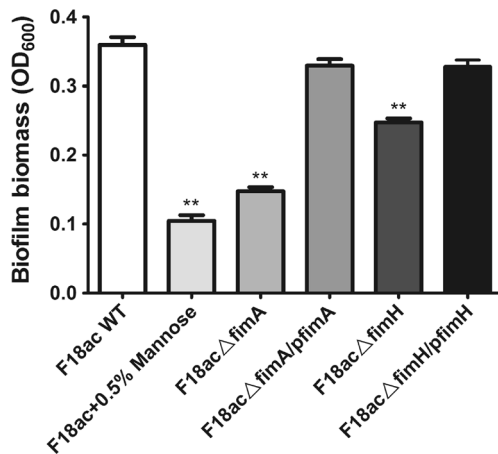
Fig. 3 Assessment of biofilm formation of WT F18ac+ ETEC, isogenic mutants, and complementary strains. Biofilm biomass was spectrophotometrically measured at OD_{600} after CV staining. The experiment was performed at least three times and six replicates were used in each single test. **Indicates statistically significant differences compared to the F18ac+ ETEC WT strain ($p < 0.01$)

Type I fimbriae affect the expression of other adhesins

To study whether deletion of the *fimA* or *fimH* genes affected the expression of other adhesins for F18ac+ ETEC, quantitative PCR (qPCR) was used to quantify the transcription of *fedF*, *fliC*, and *AIDA-I* genes using *gapA* as a normalizing internal standard. The expression of *AIDA-I* was upregulated 2.84- and 3.37-fold in F18ac△fimA and F18ac△fimH mutants, respectively (Table 3). The deletion of *fimA* resulted in 1.32- and 1.21-fold decrease in *fliC* and *fedF* expression, respectively. Deletion of the *fimH* caused 1.54- and 1.17-fold decrease in *fliC* and *fedF* expression, respectively (Table 3). In contrast to *AIDA-I*, these differences are not statistically significant.

Discussion

PWD is one of the most common diseases in piglets. Piglets develop PWD 3–10 days after weaning, which causes weight loss, slow growth, and acute death (Svensmark et al. 1989; Verdonck et al. 2002; Vu-Khac et al. 2007). To date, there are no effective prevention strategies against PWD. Therefore, gaining further insight to the pathogenesis of the disease can

be helpful to develop prevention strategies. The most important pathogens causing PWD are ETEC, *E. coli* strains producing enterotoxins. ETEC associated with PWD produce fimbriae including K88 and F18 and enterotoxins including LT and ST (Frydendahl 2002; Zhang et al. 2007). Initial attachment and adherence to the host's mucosal surface mediated by bacterial fimbriae is a prerequisite for PWD. Bacterial adherence is a complex and multifactorial process, which is often mediated by different adhesins (Nandre et al. 2016). F18ac fimbriae and flagella are the known adhesins involved in mediating F18ac+ ETEC adherence. It was demonstrated that the absence of F18ac fimbriae or flagella highly impairs the adherence ability of F18ac+ ETEC (Frydendahl 2002; Duan et al. 2013). Data from the current study suggest that type I fimbriae play roles in the adherence of F18ac+ ETEC strains to piglet epithelial cells.

Type I fimbriae are expressed by most *E. coli* strains and mediate mannose-sensitive (MS) adherence to host epithelial cells (Iida et al. 2001). This study showed that the adherence of F18ac+ ETEC to IPEC-1 and IPEC-J2 was highly inhibited with exogenous 0.5% D-mannose. However, an increase in D-mannose concentration did not lead to additional inhibition of adherence. Data from this study showed that the F18ac△fimA and F18ac△fimH mutants had significantly lower adherence rates to IPEC-1 and IPEC-J2 cells compared to those of the wild-type strain or the complemented strains. These results indicated that adherence roles are evident for F18+ ETEC type I fimbriae. However, the adherence to porcine cell lines was not completely abrogated in the type I fimbriae mutants. This may suggest that other adhesins could be involved in mediating F18ac+ ETEC adherence.

Adherence ability was not significantly different between the F18ac△fimA and F18ac△fimH mutants. FimH is the lectin and adhesin of type I fimbriae. FimH binds to receptors containing terminal mannose residues and mediates agglutination of yeast and red blood cells (Schembri et al. 2000; Stahlhut et al. 2009); therefore, FimH-mediated binding can be inhibited by exogenous D-mannose. Both F18ac△fimA and F18ac△fimH mutants lost their ability to agglutinate with yeast cells and pig erythrocytes, indicating deficient expression of fimH in these two mutant strains. However, the mechanism by which FimA deletion impairs FimH expression is still unknown. Nevertheless, the results from this study indicated that adhesin FimH of type I fimbriae also mediate

Table 3 Transcript expression of the other potential adhesin genes was measured using quantitative PCR (qPCR)

Gene	Annotation	Fold change in F18ac△fimA mutant	Fold change in F18ac△fimH mutant
FliC	Flagellin subunit of flagella	− 1.32	− 1.54
FedF	Subunit of F18 fimbriae	− 1.21	− 1.17
AIDA-I	A non-fimbriae adhesin	+ 2.84[a]	+ 3.37[a]

[a] Compared to expression levels in wild-type F18ac+ ETEC. $p < 0.05$

F18ac+ ETEC attachment to piglet intestinal epithelium cells besides F18 fimbriae and flagella.

Biofilm formation facilitates firm and irreversible adhesion to a surface, which is highly advantageous for bacteria survival (Koczan et al. 2011). Initial bacterial adherence to cell surfaces is in favor of biofilm formation. Increasing evidences suggest that type I fimbriae play attractive roles in the enhancement of biofilm formation (Nandre et al. 2012; Grzymajło et al. 2013; Kuźmińska-Bajor et al. 2015). The current study found that the F18acΔfimA and F18acΔfimH mutant strains were less effective in biofilm formation compared to the F18ac+ ETEC parent strains. This study also observed that the introduction of 0.5% D-mannose in the biofilm-inducing medium significantly reduced the biofilm formation levels of the F18ac+ ETEC parent strain. After incubation with 0.5% D-mannose, the absorbance of F18ac+ ETEC parent strains was inhibited by 69% as compared with the parent strains. These results demonstrate that type I fimbriae may play a role in enhancing F18ac+ ETEC biofilm formation in vitro. But whether type I fimbriae are directly involved in the biofilm formation process or indirectly down-regulate expression of other genes warrants further studies.

Expression of surface structures in *E. coli* is a complex and cross-regulated process involving type I fimbriae and other surface organelles, including P fimbriae, K88 fimbriae, and flagella (Holden et al. 2006; Lane et al. 2007; Zhou et al. 2013). Using RT-PCR, an up-regulation of the non-fimbrial adhesion AIDA-I in the in vitro cultures of the above *fimA* and *fimH* deletion mutants was detected, which was in contrast to the gene regulations for F18ac and flagellin expressions. AIDA-I is an auto-transporter protein expressed on the bacterial outer membrane and appears to mediate bacterial initial adherence and biofilm formation in vitro (Ravi et al. 2007). In order to save energy and survive in vivo, bacteria predominantly produce specific type fimbriae during the infection process (Croxen and Finlay 2010). For F18ac+ ETEC, specific F18ac fimbriae are the predominant adhesins. This may explain why type I fimbriae play a minor role in the F18ac+ ETEC adherence in vivo. It was found that AIDA-I and F18ac fimbriae are governed on the same plasmid (Mainil et al. 2002), but they could be regulated differently in the mutant strains. The exact mechanism of cross-regulation between type I fimbriae and other adhesion factors needs to be further investigated.

In conclusion, the present study demonstrated that type I fimbriae contribute to the adherence of F18ac+ ETEC to porcine intestinal epithelial cells and the early phases of biofilm formation in vitro. Additionally, there may be regulatory cross-links among F18ac+ ETEC adhesins, including type I fimbriae and AIDA-I adhesin. As the adherence of F18ac+ ETEC to piglet epithelial cells is considered a crucial and initial step of pathogenesis, the results from this study provide insights into the pathogenic mechanisms of the PWD-causing F18ac+ ETEC and, perhaps, helpful information for the development of new strategies against F18ac+ ETEC infections. Notwithstanding some viewpoints which considered that type I fimbriae contribute little, or even negatively, to pathogenicity in vivo (May et al. 1993; Chen et al. 2003), the significance of type I fimbriae of F18ac+ ETEC in the detailed pathogenesis of PWD remains to be elucidated in future studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Anderson GG, Palermo JJ, Schilling JD et al (2003) Intracellular bacterial biofilm-like pods in urinary tract infections. Science 301:105–107

Bien J, Sokolova O, Bozko P (2012) Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. Int J Nephrol 2012:681473

Boudeau J, Barnich N, Darfeuille-Michaud A (2001) Type 1 pili-mediated adherence of *Escherichia coli* strain LF82 isolated from Crohn's disease is involved in bacterial invasion of intestinal epithelial cells. Mol Microbiol 39:1272–1284

Casey TA, Nagy B, Moon HW (1992) Pathogenicity of porcine enterotoxigenic *Escherichia coli* that do not express K88, K99, F41, or 987P adhesins. Am J Vet Res 53:1488–1492

Chen YM, Wright PJ, Lee CS et al (2003) Uropathogenic virulence factors in isolates of *Escherichia coli* from clinical cases of canine pyometra and feces of healthy bitches. Vet Microbiol 94:57–69

Croxen MA, Finlay BB (2010) Molecular mechanisms of *Escherichia coli* pathogenicity. Nat Rev Microbiol 8:26–38

Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A 97:6640–6645

de la Fé Rodríguez PY, Coddens A, Del Fava E et al (2011) High prevalence of F4+ and F18+ *Escherichia coli* in Cuban piggeries as determined by serological survey. Trop Anim Health Prod 43:937–946

Duan QD, Zhou MX, Zhu XF et al (2013) Flagella from F18+ *Escherichia coli* play a role in adhesion to pig epithelial cell lines. Microb Pathog 55:32–38

Fairbrother JM, Nadeau É, Gyles CL (2005) *Escherichia coli* in post-weaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev 6:17–39

Frydendahl K (2002) Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Vet Microbiol 85:169–182

Grzymajło K, Ugorski M, Kolenda R et al (2013) FimH adhesin from host unrestricted *Salmonella enteritidis* binds to different

glycoprotein ligands expressed by enterocytes from sheep, pig and cattle than FimH adhesins from host restricted *Salmonella abortus-ovis*, *Salmonella choleraesuis* and *Salmonella dublin*. Vet Microbiol 166:550–557

Holden NJ, Totsika M, Mahler E et al (2006) Demonstration of regulatory cross-talk between P fimbriae and type 1 fimbriae in uropathogenic *Escherichia coli*. Microbiology 152:1143–1153

Hossain MM, Tsuyumu S (2006) Flagella-mediated motility is required for biofilm formation by *Erwinia carotovora* subsp. *carotovora*. Gen Plant Pathol 72:34–39

Iida KI, Mizunoe Y, Wai SN et al (2001) Type 1 fimbriation and its phase switching in diarrheagenic *Escherichia coli* strains. Clin Diagn Lab Immunol 8:489–495

Jouve M, Garcia MI, Courcoux P et al (1997) Adhesion to and invasion of HeLa cells by pathogenic *Escherichia coli* carrying the *afa-3* gene cluster are mediated by the AfaE and AfaD proteins, respectively. Infect Immun 65:4082–4089

Klemm P (1984) The fimA gene encoding the type I fimbrial subunit of Escherichia coli: nucleotide sequence and primary structure of the protein. Eur J Biochem 143:395–399

Klemm P, Christiansen G (1987) Three *fim* genes required for the regulation of length and mediation of adhesion of *Escherichia coli* type 1 fimbriae. Mol Gen Genet 208:439–445

Koczan JM, Lenneman BR, McGrath MJ et al (2011) Cell surface attachment structures contribute to biofilm formation and xylem colonization by *Erwinia amylovora*. Appl Environ Microbiol 77:7031–7039

Krogfelt KA, Bergmans H, Klemm P (1990) Direct evidence that the FimH protein is the mannose-specific adhesin of *Escherichia coli* type 1 fimbriae. Infect Immun 58:1995–1998

Kuźmińska-Bajor M, Grzymajło K, Ugorski M (2015) Type 1 fimbriae are important factors limiting the dissemination and colonization of mice by *Salmonella* Enteritidis and contribute to the induction of intestinal inflammation during *Salmonella* invasion. Front Microbiol 6:276

Lane MC, Simms AN, Mobley HL (2007) Complex interplay between type 1 fimbrial expression and flagellum-mediated motility of uropathogenic *Escherichia coli*. J Bacteriol 189:5523–5533

Mainil JG, Jacquemin E, Pohl P et al (2002) DNA sequences coding for the F18 fimbriae and AIDA adhesin are localised on the same plasmid in *Escherichia coli* isolates from piglets. Vet Microbiol 86:303–311

May AK, Bloch CA, Sawyer RG et al (1993) Enhanced virulence of *Escherichia coli* bearing a site-targeted mutation in the major structural subunit of type 1 fimbriae. Infect Immun 61:1667–1673

Müller CM, Åberg A, Straseviçiene J et al (2009) Type 1 fimbriae, a colonization factor of uropathogenic *Escherichia coli*, are controlled by the metabolic sensor CRP-cAMP. PLoS Pathog 5:e1000303

Murphy CN, Mortensen MS, Krogfelt KA et al (2013) Role of *Klebsiella pneumoniae* type 1 and type 3 fimbriae in colonizing silicone tubes implanted into the bladders of mice as a model of catheter-associated urinary tract infections. Infect Immun 81:3009–3017

Nandre RM, Matsuda K, Chaudhari AA et al (2012) A genetically engineered derivative of *Salmonella* Enteritidis as a novel live vaccine candidate for salmonellosis in chickens. Res Vet Sci 93(2):596–603

Nandre RM, Ruan X, Duan Q et al (2016) Antibodies derived from an enterotoxigenic *Escherichia coli* (ETEC) adhesin tip MEFA (multiepitope fusion antigen) against adherence of nine ETEC adhesins: CFA/I, CS1, CS2, CS3, CS4, CS5, CS6, CS21 and EtpA. Vaccine 34(31):3620–3625

Orndorff PE, Falkow ST (1984) Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. J Bacteriol 159: 736–744

Rausch D, Ruan X, Nandre R et al (2017) Antibodies derived from a toxoid MEFA (multiepitope fusion antigen) show neutralizing activities against heat-labile toxin (LT), heat-stable toxins (STa, STb), and Shiga toxin 2e (Stx2e) of porcine enterotoxigenic *Escherichia coli* (ETEC). Vet Microbiol 202:79–89

Ravi M, Ngeleka M, Kim SH et al (2007) Contribution of AIDA-I to the pathogenicity of a porcine diarrheagenic *Escherichia coli* and to intestinalcolonization through biofilm formation in pigs. Vet Microbiol 120:308–319

Schembri MA, Sokurenko EV, Klemm P (2000) Functional flexibility of the FimH adhesin: insights from a random mutant library. Infect Immun 68:2638–2646

Stahlhut SG, Tchesnokova V, Struve C et al (2009) Comparative structure–function analysis of mannose-specific FimH adhesins from *Klebsiella pneumoniae* and *Escherichia coli*. J Bacteriol 191: 6592–6601

Svensmark B, Jorsal SE, Nielsen K et al (1989) Epidemiological studies of piglet diarrhoea in intensively managed Danish sow herds. I. Preweaning diarrhoea. Acta Vet Scand 30:43–53

Verdonck F, Cox E, van Gog K et al (2002) Different kinetic of antibody responses following infection of newly weaned pigs with an F4 enterotoxigenic *Escherichia coli* strain or an F18 verotoxigenic *Escherichia coli* strain. Vaccine 20:2995–3004

Vu-Khac H, Holoda E, Pilipcinec E et al (2007) Serotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from piglets with diarrhoea in Slovakia. Vet J 174:176–187

Wiles TJ, Kulesus RR, Mulvey MA (2008) Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp Mol Pathol 85:11–19

Zhang W, Zhao M, Ruesch L et al (2007) Prevalence of virulence genes in *Escherichia coli* strains recently isolated from young pigs with diarrhea in the US. Vet Microbiol 123:145–152

Zhou M, Duan Q, Zhu X et al (2013) Both flagella and F4 fimbriae from F4ac+ enterotoxigenic *Escherichia coli* contribute to attachment to IPEC-J2 cells in vitro. Vet Res 44:30