RESEARCH ARTICLE

The long polar fimbriae operon and its flanking regions in bovine *Escherichia coli* O157:H43 and STEC O136:H12 strains

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This paper highlights the diversity of the *Escherichia coli* species and one of the virulence factors of these pathogens of animals and man.

Abstract

Long polar fimbriae (Lpf) are intestinal adhesins and important virulence factors of pathogenic *Escherichia coli* strains. We cloned and sequenced the *lpf2-1* operon (*lpf2ABCD)* and its flanking regions of an intimin- and Shiga toxin-negative atypical *E. coli* O157:H43 strain of bovine origin and also sequenced the *lpf2-1* operon of six additional atypical O157 bovine *E. coli* strains of various serotypes. Nucleotide sequence comparison of these *lpf* operons showed sequence conservation as they contained only four polymorphic nucleotide positions. Investigation of these O157 strains as well as 13 *E. coli* Reference Collection (ECOR) strains carrying the *lpf2-1* allele revealed high degree of sequence conservation in the *lpf2* flanking regions. The *lpf2-1* allele is also present in a bovine Shiga toxin-producing *E. coli* STEC O136:H12 strain, and *in vitro* adherence assays revealed that the absence of *lpf2-1* in this strain did not affect its host cell-binding properties. Our data indicate that *lpf2* loci are highly conserved in *E. coli* isolates; however, its role in adherence might be masked by other uncharacterized adhesins.

Introduction

Escherichia coli is an important member of the commensal intestinal microbial communities in mammals, but there are a large number of isolates, which have acquired a variety of virulence factors and are capable of causing serious diseases in humans and animals, including those classified as enterohaemorrhagic *E. coli* (EHEC; Kaper *et al.*, 2004). The frequent emergence of new isolates with new combinations of virulence genes is exemplified by the appearance of the strain responsible for the recent outbreak of haemolytic uraemic syndrome (HUS) in Germany (Mellmann *et al.*, 2011) and underlines the importance of the various possible lateral gene transfer mechanisms in the spread of virulence genes. Typical EHEC O157:H7/NM strains carry *stx* genes encoding Shiga toxin and also harbour a pathogenicity island (PAI) known as the locus of enterocyte effacement (LEE), encoding the intimin adhesin, among other virulence factors (Kaper *et al.*, 2004).

In addition to several extensively studied virulence factors carried by pathogenic *E. coli*, there are several additional factors such as the long polar fimbriae (Lpf), which represent a relatively recently described adhesin and virulence determinant in EHEC (Doughty *et al.*, 2002). The exact mechanism by which Lpf contribute to the virulence of each pathogenic *E. coli* strain is currently under investigation, but there is

well-documented evidence that Lpf promote adhesion of EHEC strains to the intestinal epithelium (Jordan *et al.*, 2004; Fitzhenry *et al.*, 2006; Torres *et al.*, 2008) as well as Lpf interact with extracellular matrix proteins (Farfan *et al.*, 2011).

Initially, two genetic variants of Lpf (Lpf1 and Lpf2) were identified in *E. coli.*, and with the availability of additional sequence data, more variants have been discovered, some of which show a degree of association with certain serotypes and/or pathogroups (Torres *et al.*, 2009). All known *lpf1* and *lpf2* operons are encoded on genomic islands, termed O islands, integrated in specific chromosomal locations (Doughty *et al.*, 2002). The Lpf variant encoded by the operon first named *lpfA$_{O113}$* (Ideses *et al.*, 2005) and later termed as allele 1 of *lpf2* (*lpf2-1*) (Torres *et al.*, 2009) is the most prevalent genetic variant of Lpf according to our present knowledge, as it has been detected in several strains from various serotypes (Toma *et al.*, 2004, 2006; Torres *et al.*, 2009; Galli *et al.*, 2010; Monaghan *et al.*, 2011). In comparison, the integration site of the *lpf2* operon is found between the genes coding for the L-glutamine/D-fructose-6-phosphate aminotransferase (*glmS*) and that of phosphate-binding periplasmic protein (*pstS*). Recently, seven *E. coli* strains of the O157 serogroup (including strain T22), isolated from healthy cattle and without key EHEC virulence factors, were found to harbour the Lpf2 variant (Sváb & Tóth, 2012). Three isolates were from the serotype O157:H43 (Tóth *et al.*, 2009), and some of these members have been in the focus of a recent study dealing with the evolution of the O157 serogroup (Iguchi *et al.*, 2011).

In the current study, we report the sequence of the *lpf2* operon and its flanking regions in strain T22, a nonsorbitol-fermenting (NSF) O157:H43 with an atypical pathotype (*stx-, eae),* monitor the presence of the operon and its flanking regions in a collection of *E. coli* strains of various serotypes carrying the *lpf2-1* allele and investigate the possible function in adherence of Lpf2 of STEC O136:H12 *in vitro* that does not possess intimin or other known adhesins found in bovine STEC strains.

Materials and methods

Bacterial strains

Escherichia coli strains used in this study are listed in Table 1. The ECOR strains were provided by Monika Kerényi (Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Hungary). Strains were grown in lysogeny broth (LB), as well as in LB and bromothymol blue agar plates. For isolation of genomic and cosmid DNA, strains were grown in tryptic soy broth (TSB).

Cosmid clone library construction

Genomic DNA was isolated from strain T22 with the phenol–chloroform method (Sambrook *et al.*, 1982) after growing overnight in TSB. The preparation of the cosmid clone library was performed with pWEB-TNC Cosmid Cloning Kit (Epicentre, Madison, WI) according to the manufacturer's instructions, with the modification that instead of mechanical

shearing, genomic DNA was subjected to a partial digestion with restriction endonuclease *Mbo*I (Fermentas, Vilnius, Lithuania). Altogether, 1000 transformant colonies were kept as cosmid library.

PCR screening for the presence of *lpf2* flanking regions

The cosmid library was screened by PCR for the presence of *lpf2*. The primers and annealing temperatures used in the reactions are listed in Table 2. The strains listed in Table 1 were screened for the presence of flanking regions.

Reverse transcription-coupled PCR on *lpfA* of *E. coli* T22

RNA was isolated from cells of a 48-h culture of *E. coli* strain T22 with RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with the modification that cells were collected with centrifugation at 13 000 *g* for 1 min. After discarding the supernatant, the bacterial pellet was processed according to the manufacturers' instructions. RNA samples were treated with Sigma DNase I Amplification Grade (Sigma-Aldrich, St. Louis, MO). The DNase-treated samples were used as templates for reverse transcription using Fermentas Maxima Reverse Transcriptase (Fermentas, Vilnius, Lithuania) according to the manufacturer's protocol. The product of this reaction was used as template in a regular PCR with the primers defined in Table 2.

Sequencing

A cosmid carrying the whole *lpf* operon was identified. DNA was isolated with the Sigma GenElute BAC DNA kit (Sigma-Aldrich, St. Louis, MO) and was sequenced at Baygen Institute (Szeged, Hungary) using the combination of Life Tech's SOLiD 4, Ion Torrent sequencing and the dideoxynucleotide methods. The products of the RT-PCR were also sequenced with the dideoxynucleotide method. Nucleotide sequence analysis and searches for open reading frames (ORFs) and homologous DNA sequences in the EMBL and GenBank database libraries were performed with the tools available from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), with Vector NTI and the CLC Bio DNA Workbench.

Construction of an *lpfA2-1* deletion mutant of STEC O136:H12

To generate an *lpfA2-1* deletion mutant of strain 187/06 (22), the *lpfA2-1* gene was replaced by a gene encoding kanamycin resistance using the lambda red recombinase system (Datsenko & Wanner, 2000). The long oligonucleotide primers used for introducing the mutation were those described earlier (Doughty *et al.*, 2002). Each primer included 20 bp of sequence homologous to the kanamycin resistance gene and 40 bp of sequence homologous to regions flanking the *lpfA2-1* gene. The kanamycin resistance gene was amplified from pKD4 by PCR. The purified PCR product (1 μg DNA) was electroporated into 187/06

Table 1 List of strains used in this study and results of the PCR scanning of the flanking regions of the *lpf2* operon

Strain	Serotype	Phylogenetic group	bgIG-phoU	phoU-pstB	pstB-pstA	pstA-pstC	pstC-pstS	pstS-lpfD	lpfA-glmS	glmS-glmU	glmU-atpC	Reference
T16	O157:H43	B1*	+	+	+	+	+	+	+	+	+	Tóth *et al.* (2009)
T22	O157:H43	B1*	+	+	+	+	+	+	+	+	+	Tóth *et al.* (2009)
T34	O157:H43	B1*	+	+	+	+	+	+	+	+	+	Tóth *et al.* (2009)
T49	O157(rough):H9	B1*	+	+	+	+	+	+	+	+	+	Tóth *et al.* (2009)
T50	O157(rough):H37r	B1*	+	+	+	+	+	+	+	+	+	Tóth *et al.* (2009)
B47	O157:NM	B1*	+	+	+	+	+	+	+	+	+	Tóth *et al.* (2009)
B54	O157(rough):H12	A*	−†	+	−†	+	+	+	+	+	+	Tóth *et al.* (2009)
ECOR7	O85:HN	A	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR23	O86:H43	A	+	+	−†	+	−	+	+	+	+	Ochman & Selander (1984)
ECOR26	O104:H21	B1	+	+	+	+	+	+	−	+	+	Ochman & Selander (1984)
ECOR30	O113:H21	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR32	O7:H21	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR33	O7:H21	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR34	O88:NM	B1	+	+	−	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR36	O79:H25	D	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR57	ON:NM	B2	−‡	−‡	+	+	−‡	−‡	−‡	−‡	−‡	Ochman & Selander (1984)
ECOR58	O112:H8	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR67	O4:H43	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR69	ON:NM	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
ECOR72	O144:H8	B1	+	+	+	+	+	+	+	+	+	Ochman & Selander (1984)
187/06 (22)	O136:H12	B2	+	+	−†	+	+	+	+	+	+	This study
C600	K12	A	−	+	+	+	−	+	+	+	+	Appleyard (1954)

HN, H antigen nontypeable; NM, nonmotile; ON, O antigen nontypeable.
*The phylogenetic group of these strains was determined in Sváb & Tóth (2012).
†These strains yielded consistently longer product with the given primer pair.
‡Strain ECOR57 yielded unspecific or weak products in all these reactions.

(22) strain, which had previously been transformed with the lambda red recombinase expression vector, pKM201. Following electroporation, transformants of 187/06 (22) were recovered at 30 °C for 2 h in LB broth and plated onto LB agar with kanamycin for overnight growth at 37 °C to induce the loss of pKM201. Kanamycin-resistant colonies were then confirmed by PCR for replacement of *lpfA2-1*. The *lpfA2-1* deletion mutant of 187/06 (22) was complemented in trans by introduction of the entire *lpf2* operon on pWSK:lpf (kindly provided by Dr. E. Hartland).

Bacterial adhesion

The potential adherence capacity of strain T22 was investigated on primary bovine kidney and testicle cells, which were kindly provided by Emília Szállás (Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary). The cells were grown to semi-confluency at 37 °C in 5% CO_2 in 24-well plates in Roswell Park Memorial Institute 1640 (RPMI 1640) medium without supplements. Prior to use, cells were washed once with PBS. Strain T22 was grown for 48 h with shaking at 200 rpm, and the cell monolayers were incubated for 5 h with *c.* 10^{10} bacteria per well. The infected monolayers were washed two times with PBS, fixed with methanol and stained with Giemsa reagent and investigated by light microscopy.

The ability of *E. coli* O136:H12 *lpfA2-1*[+] strain and its deletion mutant to adhere to Hep-2, Caco-2 and T84 cell lines was assessed as previously described (Doughty *et al.*,

Table 2 Primers used for the amplification of regions flanking the *lpf2* operon

Primer	Sequence (5′->3′)	Genes amplified	GenBank number	Position amplified	Annealing temperature (°C)	Reference
bglGfw	CCCAAGCGCTCCTGCGCTAAA	*bglG-phoU*	CU928160.2	3977599–3978492	60	This study
phoUrev	TCTGGAGTCGCTGGGCCGTC					This study
phoUfw	CGATCACGCGCTTCGCCAGA	*phoU-pstB*	CU928160.2	3978707–3979162	59	This study
pstBrev	GGTATCGCCATTCGCCCGGA					This study
pstBfw	GGACGGCCCGATAAACGCCG	*pstB-pstA*	CU928160.2	3979526–3979912	60	This study
pstArev	CAGCCGATCGCCAACCTGCC					This study
pstAfw	AGCCAGAACAGGCCGAAGGC	*pstA-pstC*	CU928160.2	3980508–3980919	59	This study
pstCrev	ATCGGCGGCATCATGCTGGG					This study
pstCfw	ACCGTAGATCGGCACCAGCG	*pstC-pstS*	CU928160.2	3981370–3981924	58	This study
pstSrev	CCAGAAAGGCGAAGATGCATGGC					This study
pstSfw	CAGACAGCGGCGCGTCAGAG	*pstS-lpfD*	CU928160.2	3982472–3983233	58	This study
lpfDrev	TGCTACCGAACCCAATACGGACAA					This study
lpfAfw	TGTCGACAATTTCACCGACGAAGTG	*lpfA-glmS*	CU928160.2	3987849–3988769	58	This study
glmSrev	GCTGCCGAGCCGTATTGAGCA					This study
glmSfw	CGTGTGTCGCCCAGCGAGTA	*glmS-glmU*	CU928160.2	3989854–3990519	58	This study
glmUrev	GGCGATGCGGAAATTGGCGA					This study
glmUfw	AGCAGATCGCCGCCGTGAC	*glmU-atpC*	CU928160.2	3991436–3992119	59	This study
atpCrev	ACGAAGCGCGAGCCATGGAA					This study
lpfA F	ACCGCTATCGATGCTGAAGG	*lpfB-lpfA*	AY057066	678–1349	63	Ideses *et al.* (2005)
lpfB R	GCGCAACATCTTCGGGAATA					
lpfC F2	CGCCGGGTTAGAAATAGATA	*lpfD-lpfC*	AY057066	3658–4421	63	Ideses *et al.* (2005)
lpfD R2	TGCCTGGTTTATTTTTGACGTA					
lpfA_inside_fw*	TCGACAGTAAATTGTGAATC	Part of *lpfA*	AY057066	233–790	50	This study
lpfA_inside_rev*	GAAGCGTAATATTATAGGCG					

*Primers used in RT-PCR for the detection of *lpfA* expression.

2002), with minor modifications. The cells were grown to semi-confluency at 37 °C in 5% CO_2 in 24-well plates (Falcon™ BD) in Dulbecco's minimal essential medium (DMEM), DMEM/F12 (Gibco, Carlsbad, CA) or MEM, depending on the cell line, with 10% or 20% (v/v) heat-inactivated foetal bovine serum, 2 mM L-glutamine and 1% (v/v) of a mixture of antibiotics/antimycotics (Gibco). Before use, the cells were washed twice with phosphate-buffered saline (PBS, Gibco) and replenished with the corresponding medium with no supplements, as it was observed previously that mannose could inhibit Lpf-mediated adherence (Farfan *et al.*, 2011). The strains were grown static in LB broth overnight at 37 °C, and tissue culture cells were incubated with *c.* 10^7 bacteria per well for 3 h at 37 °C and 5% CO_2. To quantify adherence, the infected monolayers were washed two times with PBS, and the adherent bacteria were recovered with 200 μL of 0.1% Triton X-100 in PBS and plated on LB agar plates containing the proper antibiotic. Data were expressed as the percentage of the bacterial inoculum recovered from triplicate wells and are the mean of at least two separate experiments. Statistical difference was expressed as the *P* value determined by a *t*-test analysis. The *in vitro* competition assays were performed as described above except that cells were inoculated with 5×10^6 cells each of mutant and wild-type bacteria (total number of bacteria/well 10^7 cells), and competition index (CI) was calculated.

Nucleotide sequence accession number

The sequences of the *E. coli* T22 *lpf2* operon and its neighbouring regions have been deposited in the GenBank database under accession number AHZD01000104. The sequences of the *lpf2* operons of *E. coli* O157 strains B47, B54, T16, T34, T49 and T50 (see Table 1) were deposited under accession numbers KC207119, KC207120, KC207121, KC207122, KC207123 and KC207124, respectively.

Results and discussion

Sequence characteristics of the *lpf2* operon in the atypical bovine O157 strains

We cloned and sequenced a 15.3-kb region from the genome of *E. coli* O157:H43 strain T22, including the *lpf2* operon. The sequence is deposited in GenBank under the accession number AHZD01000104. The schematic representation of the sequenced region is shown in Fig. 1. According to our knowledge, this is the first time that the allelic variant *lpf2-1* operon and its flanking regions were sequenced in a NSF strain of the serotype O157:H43. The *lpf2-1* operon itself has been detected earlier by PCR in other *E. coli* strains (Torres *et al.*, 2009; Farfan *et al.*, 2011).

In the *lpf2-1* operons of T22 and six other atypical O157 strains, there are only four positions that show polymorphism

(Supporting Information, Fig. S1). One of them is a synonymous point mutation in the gene *lpfC* of strain T49, the others produce amino acid switches in the respective genes. The *lpf2A* gene is uniformly conserved in the investigated strains, and this is also true for the majority of the strains with whole genomes available in GenBank, an exception is the *lpf2A* gene of strain 55989 (Table 3), which contains an isoleucine instead of a leucine in position 116. The *lpf2B* has an alanine instead of serine in position 99 in all the atypical O157 strains as opposed to the strains from GenBank listed in Table 3.

The *lpf2C* gene proved to be uniform in all the sequenced strains with the exception of T49, which has a cysteine in position 809 instead of tryptophan – the second polymorphism within the *lpf* operons of the atypical O157 strains. The majority of strains with sequenced genomes in Table 3 have identical lpfC to that of T22; however, three of them have polymorphisms in lpfC. Strain SE11 has a leucine in position 224 instead of an isoleucine, strain 11368 has a serine instead of a proline in position 739, and E24377A has a leucine instead of proline in position 122.

In the case of *lpf2D* gene, strain T22 has serine instead of alanine in position 341, this switch is shared with strains SE11, 55989 and 11368 and is the third polymorphism within the sequenced atypical strains. The fourth polymorphism can be observed in strains T49 and T50, which encode a leucine instead of a methionine in position 313. The nucleotide sequence comparison of the *lpf* genes of the atypical O157 bovine strains is shown in Fig. S1.

The existence of these polymorphisms indicate a similar level of variation in the otherwise conserved *lpf2* sequences. However, currently, there are no data available on the potential or actual effect of these differences on the expression and/or function of Lpf2.

The GC content of the sequenced *lpf* operons was 44%, while that of the flanking regions in T22 was 52%, close to the average GC content in the *E. coli* genome (McLean *et al.*, 1998). This fact, together with the generally conserved sequence of *lpf2* in strains of various sero- and pathotypes (Table 3), is in harmony with previous findings that *lpf2* operon is located on a genomic island (Doughty *et al.*, 2002).

Dissemination and characteristics of the flanking regions of *lpf2*

The results of the PCR scanning of previously characterized strains carrying allele *lpf2-1* are listed in Table 1. The fact that the *lpf2-1* operon is flanked by the same set of genes in the majority of the strains sequenced so far (Table 3) is further demonstrated by the results of our PCR scanning. This uniformity indicates that the site between the *pstS* and *glmS* genes served as an integration hot spot at some point during the evolution of these strains. The genetic analysis of this region was performed in an earlier study, in which the authors designed primers specific for the flanking regions, and investigated whether the site between *pstS* and *glmS* is intact or interrupted by the *lpf2* operon (Toma *et al.*, 2006). It must be noted, however, that in the case of prototypic enteroaggregative strain *E. coli* (EAEC) strain 042 (O44:H18), a Tn*21* transposon sequence is inserted between *lpfA* and *glmS* genes. This transposon element harbours transposases and genes encoding antibiotic resistance among other features (Chaudhuri *et al.*, 2010). Interestingly, four of five strains that have the closest homologues to the *lpf2-1* operon of strain T22 are commensal isolates (Table 3).

Expression of Lpf2

The RT-PCR specific for the *lpfA* genes from T22 yielded positive results confirming transcription of Lpf2 in 48-h cultures. In an earlier study with EHEC strain EDL933, one of the authors of the current manuscript found that the H-NS protein has a silencer role, while the regulatory protein Ler acts as an antisilencer during the expression of Lpf1 (Torres *et al.*, 2008). Our findings, as well as the lack of LEE (which includes the *ler* gene) in strain T22, suggest a different regulatory mechanism controlling Lpf2 expression relative to Lpf1.

Contribution of LpfA2-1 to adherence of STEC O136: H12 *in vitro*

The wide distribution of this particular allele of Lpf in pathogenic *E. coli* strains, especially in LEE-negative strains (Doughty *et al.*, 2002), underlines its potential role as an important adhesin. There are both *in vitro* (Doughty *et al.*, 2002; Newton *et al.*, 2004; Torres *et al.*, 2008; Farfan *et al.*, 2011) and *in vivo* (Jordan *et al.*, 2004) experimental evidence that Lpf enhance the adherence of intimin-positive and intimin-negative strains. Construction of an *lpf2* mutant in strain T22 resulted more challenging than expected; therefore, we created an *lpf2-1* mutant in STEC O136:H12 strain 187/06 (22), a bovine isolate that possesses the same *lpf2-1* allele as strain T22 (data not shown) and flanked with the same genes (Table 3). The role of Lpf2 in adherence of strain 187/06 (22) was

Fig. 1 Schematic representation of the genetic region from *Escherichia coli* strain T22 containing *lpf2*. The relative length of the arrows is proportional to the relative length of the genes. Arrows representing genes from the same functional cluster are filled with the same pattern. The *pst* cluster encodes a phosphate ABC transporter, *phoU* encodes a phosphate transport regulator, and the product of *bglG* is a putative transcriptional regulator. The *glm* cluster encodes an *N*-acetyl glucosamine-1-phosphate uridyltransferase, and *atpC* encodes the epsilon subunit of ATP synthase.

Table 3 *Escherichia coli* strains with whole genomes in GenBank containing continuous *lpf2* operons with high homology to the *lpf2-1* of strain T22

Strain	Serotype	Pathotype	SNPs in the Lpf operon	GenBank number	Reference
SE11		Commensal	6	AP009240.1	Oshima *et al.* (2008)
11128	O111:NM	EHEC	7	AP010960.1	Ogura *et al.* (2009)
55989		EAEC	8	CU928145.2	Touchon *et al.* (2009)
KO11		Commensal	9	CP002516.1	unpublished, JGI Project ID: (4085)738
W		Commensal	9	CP002185.1	Archer *et al.* (2011)
11368	O26:H11	EHEC	9	AP010953.1	Ogura *et al.* (2009)
IAI1		Commensal	10	CU928160.2	Touchon *et al.* (2009)
E24377A		ETEC	11	CP000800.1	Rasko *et al.* (2008)

evaluated using different tissue cultured cells lines; however, no clear differences could be observed in the quantitative adherence assays between O136:H12 and its corresponding *lpfA2-1* mutant. The strain 187/06 (22) did not show a significant reduction in the adherence neither to Hep-2 ($P = 0.10$), nor to Caco-2 ($P = 0.42$) cell lines when compared to its deletion mutant, but exhibited a significant reduction in adherence to T84 cell line ($P < 0.0002$) (Fig. 2). However, when competition adhesion assays were performed using the wild-type and its corresponding *lpfA2-1* deletion mutant, the mean CI (3.27 ± 1.35) was significantly > 1 ($P = 0.043$). This finding, where the mutant strain is adhering more than the wild type, suggested that the strain lacking Lpf might produce additional adhesion factors that provided a subtle advantage in the *in vitro* adherence assay.

In the case of strain T22, no specific adhesion could be observed in bovine testicle and kidney cell cultures (data not shown), which is in harmony with the finding that although strains possessing Lpf2 are not defective in expression, further analysis is needed to define the role of this fimbriae or other adhesion factors in these subset of strains.

In summary, we cloned and sequenced for the first time the long polar fimbriae-encoding operon and its flanking regions in an atypical, NSF O157:H43 *E. coli* strain. The Lpf operon itself is nearly identical to those of several other pathogenic and nonpathogenic strains from various serotypes and pathogroups and represents the genetic variant termed allele 1 of *lpf2*. The integration site of the operon also shows high similarity to that on the aforementioned strains. Characteristic flanking regions were also found in other O157 non-H7 strains carrying the same operon. Given the experimental evidence for the role of Lpf in adherence of *E. coli* O157:H7 and other pathogenic *E. coli* strains, it is plausible to propose that the Lpf2-1 fimbriae are important factors mediating adherence of our bovine *E. coli* strains. However, we did not observe any difference in the adherence profile of STEC 187/06 (22). Based on recently published report, we speculate that in the absence of an adhesin such as Lpf, the strains synthesized alternate surface structure as a compensatory mechanism for colonization (Lloyd *et al.*, 2012). Further, the complete regulatory mechanisms of *lpf2* still need to be fully elucidated. Finally, the highly conserved sequence and integration site of the *lpf2* operon suggest that *lpf2* loci
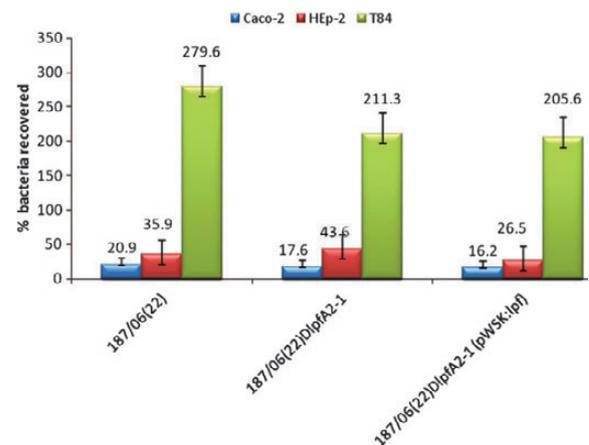

Fig. 2 Comparative chart of *in vitro* bacterial adhesion assays to Caco-2, Hep-2 and T84 cell lines. The results are expressed as the percentage of cell-associated bacteria from the original inoculum [(final CFU mL^{-1}/initial CFU mL^{-1}) $\times$ 100] and are the means $\pm$ the standard error of at least two independent experiments in triplicate wells.

belong to a conserved genomic island, and an interesting future task will be to elucidate the mechanism of genetic acquisition of this operon in pathogenic and commensal *E. coli* strains.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. (a–d) Sequence comparison of the *lpf2* operons of atypical bovine *Escherichia coli* O157 strains.