

# A Mosaic Pathogenicity Island Made Up of the Locus of Enterocyte Effacement and a Pathogenicity Island of *Escherichia coli* O157:H7 Is Frequently Present in Attaching and Effacing *E. coli*

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**Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) possess a pathogenicity island (PAI), termed the locus of enterocyte effacement (LEE), which confers the capability to cause the characteristic attaching and effacing lesions of the brush border. Due to this common property, these organisms are also termed attaching and effacing *E. coli* (AEEC). Sequencing of the EHEC O157 genome recently revealed the presence of other putative PAIs in the chromosome of this organism. In this article, we report on the presence of four of those PAIs in a panel of 133 *E. coli* strains belonging to different pathogroups and serotypes. One of these PAIs, termed O122 in strain EDL 933 and SpLE3 in strain Sakai, was observed in most of the AEEC strains examined but not in the other groups of *E. coli*. It was also found to contain the virulence-associated gene *efa1/lifA*. In EHEC O157, PAI O122 is located 0.7 Mb away from the LEE. Conversely, we demonstrated that in many EHEC non-O157 strains and EPEC strains belonging to eight serogroups, PAI O122 and the LEE are physically linked to form a cointegrated structure. This structure can be considered a mosaic PAI that could have been acquired originally by AEEC. In some clones, such as EHEC O157, the LEE-O122 mosaic PAI might have undergone recombinational events, resulting in the insertion of the portion referred to as PAI O122 in a different location.**

Certain strains of *Escherichia coli* are capable of causing diarrheal diseases in human beings and animals by colonizing the intestinal mucosa with a characteristic mechanism known as attaching and effacing (A/E) (28). Colonizing bacteria induce the effacement of epithelial cell microvilli and develop intimate contact with the cell membrane (9, 13). The *E. coli* strains that show this pathogenic property are referred as attaching and effacing *E. coli* (AEEC). AEEC can be divided into two main pathogroups: enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) (28). EHEC strains produce Shiga toxins (Stx) and cause severe human illnesses, such as hemorrhagic colitis and hemolytic-uremic syndrome (14, 30). The majority of the cases of disease worldwide are caused by strains of serotype O157:H7, but infections caused by EHEC strains belonging to serogroups other than O157, such as O26, O111, O103, and O145, have been increasingly reported (7, 14). EPEC strains do not produce Stx and are not associated with hemolytic-uremic syndrome but represent an important cause of diarrhea in children, in particular in non-industrialized countries (28), and in young animals of various species (2, 3, 33, 36, 43).

The capability to cause A/E lesions is conferred by the presence of a chromosomal genetic element defined as the locus of enterocyte effacement (LEE) (10, 23). The LEE is constituted by 41 open reading frames (ORFs) organized in five polycistronic operons: LEE1, LEE2, LEE3, *tir*, and LEE4 (24). The operons LEE1, LEE2, and LEE3 encode the components of a

type III secretion system (24), while the LEE4 operon encode proteins which are secreted by the type III secretion machinery (13). The *tir* operon contains the *eae* gene, encoding the outer membrane adhesion molecule intimin (10), and the *tir* gene, encoding the translocated intimin receptor (18). The low GC content of the LEE (10), together with other features, such as the carriage of virulence genes, the large size, and the insertion in chromosomal loci encoding tRNAs (17, 38, 42), indicates that this locus is a pathogenicity island (PAI) (4, 15, 23).

The LEE is not the only genetic element that AEEC strains have acquired by horizontal transfer. In EHEC strains, Stx genes have been transduced by bacteriophages (30), and in some strains, the enterohemolysin (*hlyA*) and katalase (*katP*) genes are harbored by a 90-kb plasmid (5, 37).

Acquisition of foreign DNA can generate new bacterial variants with new virulence properties (12, 15), and phylogenetic analyses have suggested that the gain and loss of mobile virulence elements have frequently occurred in separate lineages of pathogenic *E. coli* (34). Moreover, some pathogenic clones, such as *E. coli* O157:H7, may be more likely to acquire foreign DNA through recombination as a side effect of a defective mismatch repair system (20).

Recently, the complete DNA sequences of *E. coli* O157:H7 strains EDL 933 (32) and Sakai (16) were determined. Genetic analyses showed that the EHEC O157 chromosome contains more than 170 genomic islands which are not present in the *E. coli* K-12 sequence and that 33% of them harbor genes with unknown functions (16, 32). This large amount of foreign DNA comprises the main known virulence-associated genetic elements of this pathogen, such as the LEE and the Stx-converting phages, and could also encode additional virulence factors or other properties involved in colonization of the gastrointes-

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TABLE 1. PCR primers used in this study

Primer	Target gene	Sequence (5'-3')	GenBank accession number (nucleotide position)
O7 right probe fwd	Z0262	TCATGGCACAGACCCGTATTTCAGC	AE005199 (1391-1368)
O7 right probe rev		TTCATGTCAGGCGCGGTATTTTTG	AE005199 (941-964)
O7 internal probe fwd	Z0260	CCCAGCCGCCATTACCCACAACAT	AE005198 (10861-10838)
O7 internal probe rev		GCTTCAGCGCGTCCGTATCCAGTA	AE005198 (10339-10362)
O7 left probe fwd	Z0245	AAAAGGTCTGGCATTTCGACATTT	AE005197 (2185-2163)
O7 left probe rev		GCTGGATATCTCATCTGGATTTTTG	AE005197 (1785-1809)
O43/48 right probe fwd	Z1214	CGGTGGGCGCTTATCAGG	AE005276 (12918-12935)
O43/48 right probe rev		GGGCCATCGCGTTTTCTCA	AE005276 (13931-13914)
O43/48 internal probe fwd	<i>terA</i>	AACCGGCTGAAACCTGATGTC	AE005273 (8830-8850)
O43/48 internal probe rev		AATTGCGCCGTTTTTCGTTTAC	AE005273 (9675-9655)
O43/48 left probe fwd	Z1128	TTGCCTACAGGAAAGACACG	AE005270 (7869-7888)
O43/48 left probe rev		TGCTACGCCTCAGAATAATACC	AE005270 (8273-8252)
O43 left junction fwd	<i>clpA</i>	CGCCATACCGTCAGCCGTCTTA	AE005270 (9750-9771)
O43 left junction rev	Z1120	CATTTCCCCCGCCCTTTACT	AE005270 (409-389)
O43 right junction fwd	Z1226	GGGACCGGTGGGGATTTTCAT	AE005277 (5971-5990)
O43 right junction rev	<i>serW</i>	CCCCCTCACCGCCAGATTAT	AE005277 (6429-6410)
O48 left junction fwd	<i>ycdU</i>	GGGGGACCGCTGAAATAAATCT	AE005306 (1826-1848)
O48 left junction rev	Z1559	ACTCGCCCCGGAATGTCACTG	AE005306 (2988-2968)
O48 right junction fwd	Z1664	CTGCGGCTGCTGGCTGATG	AE005314 (3417-3435)
O48 right junction rev	<i>serX</i>	GTGAGGTGTCCGAGTGGCTGAAG	AE005314 (4286-4264)
O122 right probe fwd	Z4334	AGACCCGCCACCCAGCATGTAT	AE005528 (6644-6666)
O122 right probe rev	Z4336	CTGCGGCCCCGAAAATGAAA	AE005528 (7801-7781)
O122 internal probe fwd	Z4326	TTCAGGAAAACAAGGGGACAAATA	AE005527 (9726-9749)
O122 internal probe rev		TGCCAAGTACGCCACAATA	AE005527 (10640-10622)
O122 left probe fwd		ATACCGCAGACCCAGCCAGACCA	AE005527 (3094-3116)
O122 left probe rev		AACCCAGCGCCCCATCGTATTG	AE005527 (4328-4307)
Efa1 fwd	<i>efa1</i> 5' region	TGGGCAGAACATTTTCACCAGTTG	AJ277443 (46852-46830)
Efa1 rev		CTTTCAGGTGGGGAACCATATGGC	AJ277443 (46111-46127)
Efa1 3' fwd	<i>efa1</i> 3' region	TGCGCACAAATGACTACAGAGGAA	AJ277443 (38050-38027)
Efa1 3' rev		ATACGACCATTGAGGGGAATCAC	AJ277443 (37337-37358)
EspF fwd	<i>espF</i>	CTTCATTACTCCCTCTCGTCCGGC	AJ277443 (36069-36093)

tinal tracts of animal reservoirs or in survival through the steps of the food chain.

Some of the exogenous genomic islands described for the EHEC O157 chromosome can be considered PAIs, since they contain putative virulence genes, have a GC content lower than that of the *E. coli* chromosome, and are inserted in tRNA locus regions.

In particular, a 22-kb PAI designated O122 in strain EDL 933 (32) and SpLE3 in strain Sakai (16) and located in the PheV tRNA locus 0.7 Mb from the LEE contains the 5' region of the *efa1/lifA* gene. This AEEC-associated (19) virulence gene is involved in the capability of EHEC to adhere to CHO cells (29) and in the repression of the host lymphocyte activation response by EPEC (19). Recently, a DNA segment containing *efa1/lifA* was described for the LEE of a rabbit EPEC O15 strain, which was significantly larger (59 kb) than those in human strains (41).

In this study, we developed a set of molecular tools for investigating the presence of PAI O122 and three other putative PAIs of EHEC O157 in a panel of *E. coli* strains belonging to various pathogroups and serotypes. We show that PAI O122 is strongly associated with AEEC and that in AEEC other than EHEC O157 it contains the entire *efa1/lifA* gene. We also show that in many AEEC strains, PAI O122 and the LEE are physically linked to form a larger, mosaic PAI.

#### MATERIALS AND METHODS

**Bacterial strains.** The *E. coli* strains examined in this study were part of the culture collection of the Istituto Superiore di Sanità and included EPEC, EHEC,

enterotoxigenic *E. coli* (ETEC) (28), enteroaggregative *E. coli* (EAEC) (25, 28), and cytotoxic necrotizing factor 1-producing *E. coli* (necrotizing *E. coli* [NTEC]) (11). Many of these strains were described in previous studies (26, 27, 31). Thirty-one out of 62 EHEC strains and 29 out of 38 EPEC strains were isolated from humans, while the others were isolated from different animal species. The porcine EPEC O45 strain was kindly provided by Josée Harel, Saint Hyacinthe, Quebec, Canada. All of the AEEC strains possessed the intimin-encoding *eae* gene (31). The EHEC strains produced Stx, as assessed by the Vero cell cytotoxicity assay and PCR amplification of Stx-encoding genes (26). Reference strains E2348/69 (EPEC O127:H6) and EDL 933 (EHEC O157:H7) were also included in this study.

The ETEC strains belonged to serogroups O6, O43, O46, and O147 and included eight strains from humans and two from animal sources. The eight EAEC strains belonged to serogroups O86, O111, O126, and O128 and were isolated from human stools. The eight NTEC strains belonged to serogroups O2, O6, O15, O22, O75, and O83 and were isolated from human urinary tract infections. Five nonpathogenic *E. coli* strains negative for all of the above-mentioned virulence genes (four isolated from human stools and K-12 strain LE 392) were also included.

**Detection of the PAIs of EHEC O157.** The presence of each putative PAI was assessed by dot blot hybridization with three probes corresponding to the left, internal, and right regions of each genomic island. Probes were obtained by PCR amplification of the genomic DNA from EHEC O157 strain EDL 933 with the primer pairs described in Table 1. PCRs were performed with 50- $\mu$ l reaction mixtures containing 50 ng of template DNA, 0.2 mM deoxynucleoside triphosphates, 1  $\mu$ M each primer, and 2.5 U of *Taq* polymerase (Stratagene, Amsterdam, The Netherlands). Genomic DNA was isolated from the bacterial strains by using a NucleoSpin tissue kit (Macherey-Nagel GmbH, Duren, Germany) under the conditions indicated by the manufacturer. Five hundred nanograms of genomic DNA was loaded into each well of a 96-well vacuum manifold and transferred to nylon membranes by applying a vacuum. The filters were washed twice with 20 $\times$  SSC (1 $\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and air dried, and DNA was cross-linked by UV exposure. Hybridization, stringent washings, and detection were performed by using an enhanced chemiluminescence direct labeling and detection system under the conditions indicated in the

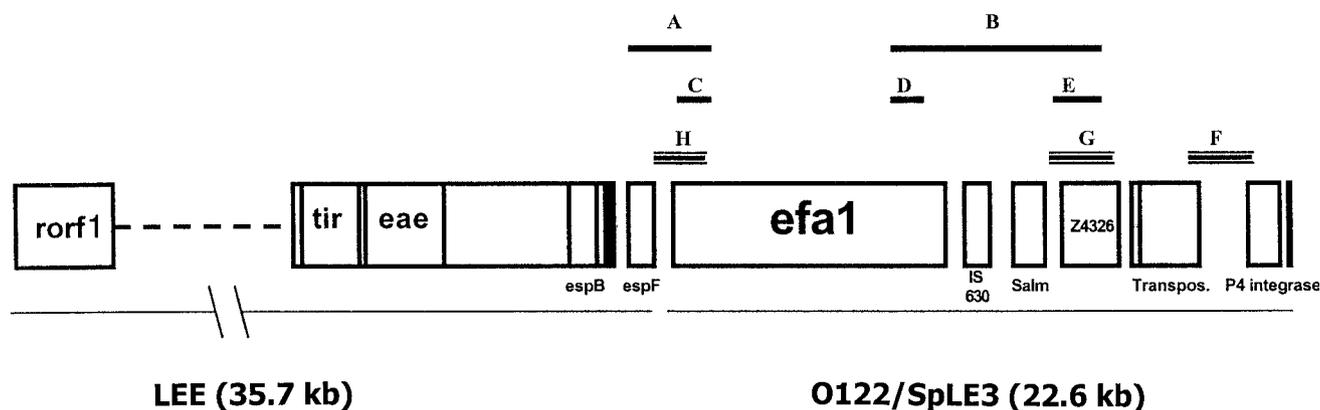


FIG. 1. Hybridization and PCR strategies used for molecular analysis of the LEE-O122 mosaic PAI in AEEC strains on the basis of the sequence of the LEE region of EHEC O26 strain 413/89-1. Sequences and locations of primers are listed in Table 1. (A) PCR product obtained with primer pair EspF fwd-Efa1 3' fwd (1.9 kb). (B) PCR product obtained with primer pair O122 internal probe fwd-Efa1 rev (6.5 kb). (C) PCR product obtained with primer pair Efa1 3' fwd-Efa1 3' rev (692 bp). (D) PCR product obtained with primer pair Efa1 fwd-Efa1 rev (725 bp). (E) PCR product obtained with primer pair O122 internal probe fwd-O122 internal probe rev (914 bp). (F, G, and H) Hybridization probes corresponding to the left, internal, and right regions of PAIO122, respectively. *rorf1*, the first ORF in the LEE region of EHEC O26 strain 413/89-1; IS, insertion sequence; Salm, *Salmonella* sequence; Transpos., transposase.

kit manual (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). A PAI was considered present when the strain reacted with at least two of the specific probes. The insertion sites for PAIs O43 and O48 were assessed by PCR amplification of the junctions with the regions shared with *E. coli* K-12 in the DNA sequence of EHEC O157 strain EDL 933 (Table 1).

**Sequence analysis.** Comparative analysis of nucleotide sequences was performed by using advanced BLAST search program 2.0 within the QBLAST system from the National Center for Biotechnology Information. The molecular maps and the positioning of ORFs were determined by using pDRAW32 software version 1.0 (ACA Clone Software).

**Molecular analysis of PAI O122.** PCR amplification was used to detect the presence of the *efa1/lifA* gene and ORF Z4326, encoding, respectively, adherence factor Efa1 (29) and a protein 58% homologous to enterotoxin ShET2 of *Shigella flexneri* (6). Since the coding sequence of *efa1/lifA* is about 10 kb long, the 3' and 5' regions of the gene were amplified separately.

The primer sequences and locations are listed in Table 1. PCRs were carried out under the conditions described above.

The location of *efa1/lifA* in PAI O122 was assessed by amplification of the region between this gene and ORF Z4326 by using primers Efa1 rev and O122 internal probe fwd (Table 1 and Fig. 1). The expected PCR product, based on the EHEC O157 strain EDL 933 genomic sequence, was 6.5 kb. The amplicons were obtained by using Herculase *Taq* polymerase (Stratagene, La Jolla, Calif.) under the conditions indicated by the supplier.

The primer pair EspF fwd-Efa1 3' fwd (Table 1 and Fig. 1) was used to assess whether the *efa1/lifA* gene was adjacent to the *espF* gene, which corresponds to the last ORF of the LEE. Since the amplicon was expected to be 1.9 kb, PCRs were performed by using the Herculase enzyme.

## RESULTS

Besides PAI O122, putative PAIs O7, O43, and O48 present in the sequences of EHEC O157 strain EDL 933 (32) and EHEC O157 strain Sakai (16) were chosen for investigation. PAIs O43 and O48 are two identical copies of the same 88-kb DNA fragment inserted in the *serW* and *serX* tRNA loci, respectively (16, 32). PAIs O7 (35.4 kb) and O122 are inserted in the *aspV* and *pheV* tRNA loci, respectively (16, 32).

**Distribution of the PAIs of EHEC O157 in AEEC strains.** The presence of the PAIs in 133 *E. coli* strains belonging to different pathogroups and serogroups was investigated. The AEEC strains included 23 EHEC O157, 40 EHEC non-O157, and 39 EPEC strains. Thirty-one *E. coli* strains belonging to different pathogroups (ETEC, EAEC, and NTEC) or exhibiting none of the considered virulence factors were examined for

comparison. For each PAI, genomic DNA was hybridized with three probes specific for the left, internal, and right regions of the island. To distinguish between PAIs O43 and O48, all of the positive isolates were subjected to amplification of the junction regions to identify the insertion sites. The results are shown in Table 2. As expected, PAI O122 was found in all of the EHEC O157 strains investigated and was also very common in both EHEC non-O157 strains (87.5%) and EPEC strains (89.7%). It appeared to be unique to AEEC, since it was not detected in any of the strains belonging to the other pathogroups.

PAI O7 was found in all of the EHEC O157 strains and in about half of the EHEC non-O157, EPEC, EAEC, and ETEC strains examined but not in NTEC and nonpathogenic *E. coli* strains. PAIs O43 and O48, in combination or alone, were observed in all of the EHEC O157 strains, in 80.0% of the EHEC non-O157 strains, and in 56.4% of the EPEC strains examined. These PAIs were also present in ETEC strains (10.0%) and NTEC strains (37.5%) but not in EAEC and nonpathogenic *E. coli* strains.

**Molecular analysis of PAI O122 in various AEEC strains.** The sequence data available for EHEC O157 strain EDL 933 indicate that, beside the 5' region of *efa1/lifA*, PAI O122 contains another putative virulence gene, ORF Z4326, coding for a protein 38% homologous to enterotoxin ShET2, encoded by the *senA* gene of *S. flexneri* (6). Therefore, a PCR approach was developed to investigate the presence of *efa1/lifA* and ORF Z4326 or portions of their sequences in AEEC strains. Since the entire coding sequence of the *efa1/lifA* gene was about 10 kb (29), two primer pairs able to amplify the 5' and 3' regions were used to assess its presence (Table 1). All 40 PAI O122-negative strains were negative in both the *efa1/lifA*- and the ORF Z4326-specific PCR assays. The results obtained with the 93 PAI O122-positive strains are shown in Table 3. ORF Z4326 was detected in all of the strains examined. As expected from the strain EDL 933 sequence, all of the EHEC O157 strains lacked the 3' end of the *efa1/lifA* gene. The same result was obtained for two of the four EHEC O145 strains examined.

TABLE 2. Distribution of EHEC O157 putative PAIs in *E. coli* strains

<i>E. coli</i>	Serogroup	No. of strains				
		Examined	Positive for:			
			O#7	O#43	O#48	O#43 + O#48
EHEC	O18	2	0	0	0	0
	O26	12	1	8	2	2
	O45	3	3	0	0	0
	O86	1	0	1	0	0
	O103	3	3	2	0	0
	O111	8	8	1	0	7
	O118	3	0	1	1	1
	O121	1	1	1	0	0
	O123	1	0	0	0	1
	O128	1	1	0	0	0
	O145	4	0	4	0	0
	O152	1	1	0	0	0
	O157	23	23 <sup>a</sup>	0	6	17 <sup>a</sup>
EPEC	O26	9	2	2	1	4
	O45	1	1	0	0	0
	O55	4	3	1	0	0
	O86	2	0	1	0	0
	O103	2	2	0	0	0
	O111	3	3	1	1	1
	O114	1	1	0	0	0
	O125	3	0	2	0	0
	O127	5	0	1	0	0
	O128	6	3	3	1	0
	ND <sup>c</sup>	3	1	1	1	1
EAEC		8	4	0	0	0
ETEC		10	5	1	0	0
NTEC		8	0	3	0	0
Nonpathogenic <sup>d</sup>		5	0	0	0	0

<sup>a</sup> Including reference strain EDL 933.

<sup>b</sup> Including reference strain E2348/69.

<sup>c</sup> ND, not determined.

<sup>d</sup> Strains showing no virulence genes associated with the other pathogroups.

Conversely, the complete sequence of the *efa1/lifA* gene was found in most (58 strains) of the EHEC non-O157 and EPEC strains. Strains of EPEC O125 (two strains examined), EPEC O127 (three out of five strains) EPEC O128 (three out of five strains), and EHEC O86 (one strain) represented a few exceptions.

***efa1/lifA* is located in PAI O122 in most AEEC strains.** To confirm that *efa1/lifA* is located in PAI O122 in AEEC strains other than EHEC O157 strains, we designed a PCR assay able to amplify the sequence between the 5' region of *efa1* and ORF Z4326 of strain EDL 933. The expected 6.5-kb PCR product was obtained from 66 out of the 83 *efa1/lifA*-positive strains (Table 3), indicating that the gene is located in PAI O122 regardless of the AEEC pathogroup or serotype.

**PAI O122 and LEE are contiguous in many EHEC and EPEC strains.** A recent report described a 15-kb DNA region flanking the LEE in a rabbit EPEC O15:H- strain (41). That region contained both *efa1/lifA* and ORF Z4326 (*senA*). Moreover, two identical genes are located in a DNA fragment contiguous to the LEE in EHEC O26:H- strain 413/89-1 (GenBank accession no. AJ277443).

These observations prompted us to develop a PCR strategy to investigate the existence of a similar LEE-O122 PAI in other AEEC clones. Based on the sequence of EHEC O26 strain 413/89-1, a primer pair able to amplify the 1.9-kb region between the *espF* in the LEE and the 3' region of *efa1/lifA* in PAI O122 (Fig. 1A) was used to analyze the 58 AEEC strains possessing the entire *efa1/lifA* gene. The link between the LEE and PAI O122 was shown in 15 EHEC non-O157 strains belonging to five serogroups and 10 EPEC strains belonging to five serogroups (Table 3). The PCR product obtained was of the expected size (1.9 kb) in 23 strains, but a larger product (4.5 kb) was obtained in two EHEC O145 strains.

TABLE 3. PCR analysis of PAI O#122 in AEEC strains

<i>E. coli</i>	Serogroup (intimin type)	No. of strains with the indicated PCR profile	PCR amplification (+) or no amplification (-) of:				
			ORF Z4326 <sup>a</sup>	Efa1-5 <sup>1b</sup>	Efa1-3 <sup>1c</sup>	Efa1-Z4326 <sup>d</sup>	EspF-Efa1 <sup>e</sup>
EHEC	O26 (β)	1	+	-	-	ND <sup>f</sup>	-
	O26 (β)	1	+	+	+	-	-
	O26 (β)	4	+	+	+	+	+
	O26 (β)	6	+	+	+	-	+
	O45 (β)	1	+	+	+	+	-
	O45 (ε)	1	+	+	+	+	-
	O86 (γ)	1	+	-	-	ND	-
	O103 (ε)	3	+	+	+	+	-
	O111 (γ <sub>2</sub> )	1	+	+	+	-	-
	O111 (γ <sub>2</sub> )	1	+	+	+	-	-
	O111 (γ <sub>2</sub> )	5	+	+	+	+	-
	O111 (γ <sub>2</sub> )	1	+	+	+	+	+
	O118 (β)	2	+	+	+	+	+
	O118 (β)	1	+	+	+	+	+
	O121 (ε)	1	+	+	+	+	-
	O123 (β)	1	+	+	+	-	+
	O145 (γ <sub>1</sub> )	2	+	+	-	+	-
	O145 (γ <sub>1</sub> )	2	+	+	+	+	+ <sup>g</sup>
	O157 (γ <sub>1</sub> )	23 <sup>h</sup>	+	+	-	+	-
EPEC	O26 (β)	4	+	+	+	+	+
	O26 (β)	2	+	+	+	+	-
	O26 (β)	2	+	+	+	-	+
	O26 (β)	1	+	+	+	-	-
	O45 (β)	1	+	+	+	+	-
	O55 (γ)	2	+	+	+	+	-
	O55 (γ)	2	+	+	+	-	-
	O55 (α)	2	+	+	+	-	-
	O103 (β)	2	+	+	+	+	-
	O111 (β)	2	+	+	+	+	-
	O111 (β)	1	+	+	+	+	+
	O114 (β)	1	+	+	+	+	+
	O125 (α)	2	+	-	-	ND	-
	O127 (γ)	3	+	-	-	ND	-
	O127 (α)	1	+	+	+	-	-
	O127 (α)	1 <sup>i</sup>	+	+	+	+	+
	O128 (β)	1	+	-	-	ND	-
	O128 (γ)	2	+	-	-	ND	-
	O128 (β)	1	+	+	+	+	-
	O128 (β)	1	+	+	+	-	+
	ND (β)	1	+	+	+	+	+
	ND (α)	1	+	+	+	+	+
	ND (β)	1	+	+	+	+	-

<sup>a</sup> E in Fig. 1.

<sup>b</sup> 5' Region of *efa1/lifA* (D in Fig. 1).

<sup>c</sup> 3' Region of *efa1/lifA* (C in Fig. 1).

<sup>d</sup> Linking region between the 5' region of *efa1/lifA* and ORF Z4326 (B in Fig. 1).

<sup>e</sup> Linking region between the LEE and PAI O#122 (A in Fig. 1).

<sup>f</sup> ND, not determined.

<sup>g</sup> The amplicon size was 4.5 kb.

<sup>h</sup> Including reference strain EDL 933.

<sup>i</sup> Reference strain E2348/69.

## DISCUSSION

Genomic plasticity has a primary role in the positive selection of organisms, which have to compete for the colonization of ecological niches and/or for the exploitation of limited resources. In bacteria, genomic plasticity is largely enhanced by horizontal gene transfer, a powerful tool in microbial evolution (1). Large DNA fragments containing virulence-associated genes, referred to as PAIs (4, 15), can be exchanged between different bacterial species, and their acquisition can generate new pathogenic variants. PAIs often carry genes derived from plasmids and phages, considered PAIs precursors, which have been assembled together as cointegrated structures and then stabilized by selective pressure through the inactivation of mobility and recombination genes (22). Several authors have suggested that this patchwork model has been the basis for the emergence of new pathogenic clones (8, 21, 35). EHEC O157 can be considered a good example of such a model of evolution. The analysis of its genome sequence (16, 32) has shown that almost 20% of the chromosome contains foreign DNA absent from the chromosome of *E. coli* K-12 and that this foreign DNA probably has been acquired from other bacterial species through horizontal gene transfer (34). Like the LEE, other regions of this foreign DNA can be considered putative PAIs, since they carry virulence-associated genes and are located within tRNA loci (16, 32).

In this report, we have shown that some of the putative PAIs of EHEC O157 are present in other AEEC clones and in *E. coli* strains belonging to other pathogroups. In particular, our results indicated that PAIs O7, O43, and O48 are quite common among pathogenic *E. coli*, since they have been detected in strains belonging to EHEC, EPEC, EAEC, ETEC, and NTEC pathogroups.

Conversely, the presence of PAI O122 seems to be a peculiar feature of AEEC strains, since it was detected in most of the EHEC and EPEC strains but not in the other groups of *E. coli* examined. PAI O122 of EHEC O157 strains (16, 32) contains the 5' region of the virulence-associated gene *efa1/lifA* (19, 29). In this report, we show that PAI O122 carried by AEEC strains other than EHEC O157 contains the entire gene. *efa1/lifA* was recently the object of several studies. Nicholls et al. (29) showed that it confers a sevenfold increase in the ability of EHEC O111:H- strain E45035 to adhere to CHO cell monolayers compared to the ability of a defective *efa1* mutant of the same strain. A role in the adherence properties of EHEC O157 strain Sakai was also hypothesized for the 5' region of *efa1/lifA* (40). Accordingly, the presence *efa1/lifA* in non-O157 EHEC strains has been associated with the capability of colonizing the intestinal tract of cattle and of inducing diarrhea in young calves (39). Finally, Klapproth et al. (19) showed that *efa1/lifA* is involved in the repression of host interleukins by EPEC strain E2648/69. Beside *efa1/lifA*, the presence in PAI O122 of at least another putative virulence gene, such as ORF Z4326 (*senA*), and its conserved structure in the vast majority of AEEC strains support the possible role of this PAI in the pathogenesis of AEEC infections.

The finding that PAI O122 is strongly associated with AEEC is in good agreement with a previous report from Klapproth et al. (19), who described the presence of *efa1/lifA* in AEEC but not in other *E. coli* pathogroups. This strong association with

AEEC strains could be due to a physical link between PAI O122 and LEE that is recognizable in the chromosomes of EHEC and EPEC strains belonging to many serogroups. Evidence suggesting such a link was already present in the literature. A DNA sequence containing both the *efa1/lifA* gene and ORF Z4326 (*senA*) has been described for the rabbit EPEC O15 strain 83/39 LEE, which is significantly larger than those previously described (41). Moreover, a DNA sequence from EHEC O26:H- strain 413/89-1 in which the LEE is contiguous to a region similar to PAI O122 has been released in GenBank under accession number AJ277443. However, none of these DNA regions had been recognized as PAI O122 of EHEC O157 strains. Using a PCR strategy based on the amplification of the region spanning *espF* in the LEE and *efa1/lifA* in PAI O122, we showed that the presence of the LEE-O122 mosaic PAI is not restricted to EHEC O26 and EPEC O15 strains, since it was identified in another 25 EHEC non-O157 and EPEC strains belonging to eight different serogroups. The strains found negative in this PCR assay may have the two islands inserted in different chromosomal sites, as in EHEC O157 strains EDL 933 (32) and Sakai (16), or may simply have differences in the sequences of the primer targets. We cannot exclude the possibility that in some strains, the region spanning *espF* and *efa1/lifA* was too large to be amplified, since variability in the size of this region was observed.

As far as the origin of the LEE-O122 mosaic PAI is concerned, it is interesting that in both of the EHEC O157 strains that have been sequenced so far (16, 32), the 3' region of PAI O122 contains a duplication of about 2,000 bp of the 3' region of the LEE of the same strains. Together with our results, this observation suggests that the LEE and PAI O122 may have been originally acquired as a unique large PAI and that in some clones, such as EHEC O157, they separated later on, following events of chromosomal rearrangement. This hypothesis is also supported by the presence of the LEE-O122 mosaic PAI in strains belonging to all of the AEEC clonal lineages other than EHEC O157: EPEC1, EHEC 2, and EPEC 2 (42). However, we cannot exclude the possibility that the two PAIs had separate origins and that recombination events elicited their fusion in some AEEC clones.

The existence of large PAIs containing the 34-kb core region of the LEE sufficient to induce the A/E phenotype has been described already for EHEC O103 (17) and O26 (GenBank accession number AJ277443) strains and for two rabbit EPEC O103 and O15 strains (41). This report identifies PAI O122 of EHEC O157 strains as a tessera of at least two of those PAIs and describes the presence of the LEE-#O122 mosaic PAI in strains belonging to several AEEC clones. In some other clones, the mosaic PAI may have undergone recombination events, resulting in the insertion of parts of the mosaic in different tRNA loci. Further studies are needed to clarify the structures of the mosaic LEE PAIs and their contributions to the pathogenesis of AEEC infections.

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