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Prevalence of Iron Transport Gene on Pathogenicity-Associated Island of Uropathogenic *Escherichia coli* in *E. coli* O157:H7 Containing Shiga Toxin Gene

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Uropathogenc *Escherichia coli* (UPEC) CFT073 has a pathogenicity-associated island (PAI_{CFT073}), which causes pyelonephritis and cystitis. Using PCR method, we found the *prrA* gene of PAI_{CFT073} in *E. coli* O157:H7 EDL933. Further detailed PCR screening of 38 open reading frames, the right and left junction sequences of PAI_{CFT073}, revealed that it is the *prrA-modD-yc73-fepC* gene cluster but not the PAI_{CFT073} present in *E. coli* O157:H7 EDL933. A rapid preliminary analysis suggested that the *prrA-modD-yc73-fepC* gene cluster of the PAI_{CFT073}, is present in 43 strains of *E. coli* O157:H7 containing Shiga toxin (Stx) gene but absent in 19 strains of *E. coli* O157:H7 without Stx gene. A strict co-occurrence of the *prrA-modD-yc73-fepC* gene cluster and Stx genes was observed, regardless of their origin. The *prrA-modD-yc73-fepC* gene cluster encode proteins probably involved in iron uptake system, which strongly suggests the importance of iron metabolism in the Stx-mediated virulence. In addition, the *prrA-modD-yc73-fepC* gene cluster may be used as a diagnostic marker to distinguish *E. coli* O157:H7 strains containing Stx gene from that without Stx gene, and possibly to quickly detect other pathogenic gram-negative bacteria containing the Stx gene.

Uropathogenic *Escherichia coli* (UPEC) strains produce hemolysin, P fimbriae, and aerobactin; exhibit serum resistance, and are encapsulated (10, 14). These features, usually absent from the typical fecal strains, imply the presence of a unique set of virulence determinants in UPEC strains, which are different from the virulence determinants of diarrheagenic *E. coli*. Many of these virulence determinants are encoded by gene clusters located on the pathogenicity-associated islands (8). Recently, Guyer et al. described the pathogenicity-associated island (PAI_{CFT073}), which contains 44 open reading frames (ORFs). Among them, 4 encode the hemolysin, 11 encode P fimbriae, and 19 show no homology to UPEC J96 or *E. coli* K-12 entries. Four genes (*prrA, modD, fepC*, and *yc73*), located on the PAI_{CFT073} near the left junction, encode proteins homologous to the TonB-dependent outer membrane receptor, the ATP-binding subunits of the molybdate transporter (ModD), the ferric enterobactin transport ATP-binding protein (FepC), and a similar *Haemophilus influenzae* yc73 protein (4). Near the right junction, *R1* and *R2* genes encode an apparent antiterminator with homology to a gene in the *sac* operon of *Bacillus subtilis*, and a homolog of the maltose- and glucose-specific component II (5).

Enterohemorrhagic *E. coli* (EHEC) O157:H7 is a novel and increasingly important class of enteric pathogens causing intestinal and renal disease, such as hemorrhagic colitis and hemolytic-uremic syndrome. During the last 10 years, it has caused numerous sporadic causes and several massive outbreaks (9, 15, H. Watanabe, A. Wada, Y. Inagaki, K. Itoh, and K. Tamura, Letter, Lancet **348:**831–832, 1996). The major virulence factors are Shiga toxins (Stx), which are responsible for death and many other symptoms in patients (12). We demonstrate here that the *prrA-modD-yc73-fepC* gene cluster of the PAI_{CFT073} is present exclusively in *E. coli* O157:H7 containing Stx gene but is absent from *E. coli* O157:H7 without the Stx gene.

At the initial study to screen the published pathogenicity islands of gram-negative bacteria in diarrheagenic *E. coli*, we happen to identify *prrA* gene of PAI_{CFT073} in *E. coli* O157:H7 EDL933. In order to prove whether *prrA* homologous gene only or PAI_{CFT073} is present in *E. coli* O157:H7, screening for PAI_{CFT073} genes by PCR method was conducted. To set up the PCR conditions for a specific detection of the PAI_{CFT073} genes and its boundary sequences, various primers were designed according to the published sequences (5) and are presented in Table 1, including the *hlyABCD* for hemolysin, *papABCDFHIJK* for P pilus, and the genes related to insertion sequences, transposons, and hypothetical proteins, including *Hp1-4* and *R1-16* (Table 1). *E. coli* CFT073, used as a positive control, was isolated from the blood and urine of a woman with acute pyelonephritis (5). Laboratory strain *E. coli* HB101 was used as a negative control. *E. coli* O157:H7 EDL933 was used as reference strain to study in detail the presence of PAI_{CFT073} genes of *E. coli* O157:H7 strains containing the Stx gene. PCR was performed with 30 cycles of reaction composed each of a denaturing step at 94°C for 1 min, an annealing step at the temperature as indicated in Table 1, and an elongation step at 72°C for various times (see Table 1), as well as one final extension step at 72°C for 10 min.

Under the conditions used, 41 PAI_{CFT073} genes were successfully amplified with the expected sizes for UPEC CFT071. Interestingly, 25 of 41 primer pairs, including primers for junc-

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TABLE 1. Primers used for amplifying PAI_{CFT073} genes PCR products obtained in *E. coli* O157:H7 EDL933 and UPEC CFT073[a]

Gene(s)	Orientation	Primer sequence	Size (bp)	Annealing temp (°C)	Extension time (min)	Presence of gene in[b]:	
						EHEC EDL933	UPEC CFT073
O241 plus *prrA* (left joint)	F	CTGGTGGTGTCATACGCTAA	1,654	55.6	1	−	+
	R	GTAACGCTGTCGGAAGAGGC					
prrA	F	ATGGTGTTGATGGGCTGGC	479	55	1	+	+
	R	CCCTGAAAAGTCGGCTGTATC					
modD	F	TGCCTGTTGCGGACTAAAT	378	55	1	+	+
	R	GTGCTGGAGTGGAGTTTGC					
yc73	F	GCGAAGCCGTGCCTGATTAT	437	56	1	+	+
	R	CAGACCTTTACCTTCAGCAA					
fepC	F	TACCTGGATAATGCTGTCGG	347	55	1	+	+
	R	ATGGTGTTGATGGGGCTG GC					
*HP1**	F	AGTGGCTCAGGCTCTCATTT	506	56.3	1	+	+
	R	TGGCATCATCGTTGGTCGTG					
*HP2**	F	CGTATTTTTCAGCGACTCCT	181	51.3	1	+	+
	R	CACTTTAGGACAATGGGTTA					
*HP3**	F	TCGTTGCTGCCTCCTGTGAA	216	54.9	1	+	+
	R	GCTGATACTGAGGGTTTCCG					
*HP4**	F	CGGCCATGTTTTCATTTTCC	222	58.7	1	+	+
	R	ATGGTCAGGGAGGTCAGCAG					
hlyC	F	CCAGTTCCCCATTACACAGA	311	51.4	1	−	+
	R	CACCCTGATGGCTCTGAATA					
hlyA	F	ATTAGTCCCCTCTCATTCCT	1,100	52.1	2	−	+
	R	ATTAGTCCCCTCTCATTCCT					
hlyB	F	GTTGTGGTGGTGTTTGAGAT	111	52.3	1	+	+
	R	TGCCAGGATTAGCCAGTGAA					
hlyD	F	AGGCTGGAACAAACTCGGTA	755	52.7	1	−	+
	R	TATCGGGTGTAAGGAAAGGC					
papG	F	TTTGCGAGTGGAGTGTATTT	622	50.7	1	−	+
	R	TACCTAACCCAACCGAAAAT					
papF	F	ATCGTTGCTTCTGACATCGG	311	52.3	1	−	+
	R	TTGACATTCCTTTTCCCTGA					
papE	F	GAGTCAAAATGGAAATCACG	204	48.7	1	−	+
	R	AAAGTTATCGCAGTCCCAAT					
papK	F	CGCTCTTTTACTGTTTGCCG	466	57.8	1	−	+
	R	ACTCTTCGTCCGCAGGGCTT					
papJ	F	TCCATACTTTCCTGCGGGCT	370	56.1	1	−	+
	R	ATAACAAGATGGTCACAGCC					
papD	F	ACAAACAACTGCCCTATCTT	424	52.5	1	−	+
	R	TCACCTTCCTCTGCCTGCTT					
papC	F	TTATCTGTTCCGTGCCATTC	769	57.2	1	−	+
	R	TTCCCGACTGCTGTAATCAT					
papH	F	TTGGCTGTGTGTTTGTTCAT	461	54.9	1	−	+
	R	CGCTTCTTCATTACCCGTCA					
papA	F	ATGCTGCTCCAACTATTCCA	393	53	11	+	+
	R	CGTTTTCACCATCTTTCAGG					
papB	F	GAAGTCATCAGTCGGTCAGG	254	52.6	1	−	+
	R	GCAAGAGCATTCAGCCGTAT					
pap1	F	TTCAAAAACCAGTATGTCGC	140	52.2	1	−	+
	R	GGAGGGAAAACCGCAGAAAT					
*R15**	F	CCAGCCTTCCCAGCAATCGT	256	57.0	1	+	+
	R	GGCACCATCCATCACAGCGA					
*R14**	F	ACCCTGATTCCTTCCCGTAA	164	52.1	1	+	+
	R	TATTACCATTGTCAGCAGCA					
*R13**	F	AACTCCGCCTTCGCAAAATA	266	57.1	1	+	+
	R	CGGGCAGTTCGTATGGTTCT					
*R12**	F	CATCTCTCCCAGTCATTACG	207	54.0	1	−	+
	R	CCCTGTTGAAAGTTGGCGTC					
R11	F	GAGGCGTATTGTTATTGTTG	253	50.7	1	−	+
	R	CTTCTGATTGGTAGGCTTGC					
R10	F	ATTGTCGCCCTTGGTCTCAT	222	51.3	1	−	+
	R	GGCAGTTCCATCAAGTTTAT					
R9	F	TAGTTATTCTTCGCCTGTCT	352	50.8	1	−	+
	R	TATTTCAGCAGGACACTACC					
R8	F	GTTGGGGTCTCAGGCACACT	200	54.8	1	−	+
	R	GGCAGCACAGGAAGCGGAAT					

Continued on following page

TABLE 1—*Continued*

Gene(s)	Orientation	Primer sequence	Size (kb)	Annealing temp (°C)	Extension time (min)	Presence of gene in[b]:	
						EHEC EDL933	UPEC CFT073
R7	F	GTGGCGGTTGTGTTGTTATC	173	55.1	1	−	+
	R	TGTCAGCCTCTACGAAACGC					
R6*	F	GTTGTGCTGGGTGGTGAGAG	369	56.6	1	+	+
	R	CTGATTGTTACGGTTGTGCC					
R5	F	CGGCAACTCTGTGAAACGAC	482	56.4	1	−	+
	R	CTTGTTACTGCCTTCGCTGT					
R4*	F	TCCTCAGCAAATACCGACCA	683	50.7	1	+	+
	R	TGGCTCTCTTCCGTCAATGC					
R3	F	GGATAACCAATAGCAGAACA	716	48.7	1	−	+
	R	CCCAGTGTGATGTATTCTAT					
R2(malX)	F	GCCGATAATGACTTGTAGGG	504	53.5	1	−	+
	R	CCACTGCTGTTTGTCTTCCA					
R1	F	GCGACAACTCAATAATCCGT	482	50.0	1	−	+
	R	TGGACAGGAGGTTATCATTT					
R1 plus *R2*	F	AGCCTTTCTGTTTTGAGCAT	1,525	51.0	2	−	+
	R	TCGCTACTATTGATTCTTGC					
R1 plus *f447* (right joint)	F	CCGCAAGAATCAATAGTAGC	564	50.7	1	−	+
	R	CTGGCGAGAAGGGGATAATG					

[a] The genes are listed in the order of their position on PAI_{CFT071}. The PCR was performed by using the forward primer (F) or the reverse primer (R), with the annealing temperature and the extension time as indicated.
[b] The presence (+) or absence (−) of the genes listed in column 1 is indicated for each strain.

tion regions, did not yield any fragment for EDL933, including the primes for *hlyACD* and *papBCDFHIJK*. However, 15 genes were amplified in the reference Stx-positive strain *E. coli* O157:H7 EDL933, including *HP1-4, R13-15, R6, R4, hlyB*, and *papA* (Table 1). It is reasonable that most of the homologous sequences are present in *E. coli* K-12. The R4 homologous gene, *iha*, has been identified in *E. coli* O157:H7 (18). The HP1 and HP2 represent the IS*600* hypothetical 31- and 11-kDa proteins HP3 and HP4 are hypothetical proteins identified in *E. coli* K-12. R15, R14, R12, and R6 are related to insertion sequences and transposons. R13 is homologous to the 12.7-kDa protein of *E. coli* K-12 (5). We cannot explain why the *papA* and *hlyB* fragments were synthesized in *E. coli* O157:H7 EDL933 at present time.

The boundary sequences of PAI_{CFT073} were also analyzed. The *prrA* gene is inside the left junction, next to *L4*. Just inside the right junction, the *R1* gene is linked to *f447* (5). Both gene *L4* and gene *f447* are sequences of *E. coli* K-12. The primers

TABLE 2. Prevalence of *prrA-modD-yc73-fepC* genes in *E. coli* O157:H7 strains with or without the Stx gene[a]

Strain group and source	No. of strains tested	No. of strains								
		hlyA[b]	*stx1*	*stx2*	*exe*	*prrA*	*modD*	*yc73*	*fepC*	*R1* plus *R2*
Control										
Positive control, UPEC CFT073	1	1−	1−	1−	1−	1+	1+	1+	1+	1+
Negative control, *E. coli* HB101	1	1−	1−	1−	1−	1−	1−	1−	1−	1−
E. coli O157:H7 strains containing Stx gene										
Patients (United States)	4	4+	3+1−	3+1−	4+	4+	3+1−	2+2−	4+	4−
Patients (China)	5	5+	5+	5+	5+	5+	5+	5+	5+	5−
Patients (Japan)	6	6+	6+	4−2+	6+	6+	5+1−	5+1−	6+	6−
Pigs (Jiangsu Province, China)	7	7+	7−	7+	7+	7+	4+3−	7+	7+	7−
Chicks (Jiangsu Province, China)	5	5+	1+	5+	5+	5+	4+1−	5+	5+	5−
Goats (Jiangsu Province, China)	16	16+	16−	16+	15+1−	16+	15+1−	16+	16+	16−
E. coli O157:H7 strains without Stx gene										
Pigs (Jiangsu Province, China)	6	6−	6−	6−	6−	6−	6−	6−	6−	6−
Pigs (Fijian Province, China)	4	4−	4−	4−	4−	4−	4−	4−	4−	4−
Chicks (Jiangsu Province, China)	4	4−	4−	4−	4−	4−	4−	4−	4−	4−
Goats (Jiangsu Province, China)	5	5−	5−	5−	5−	5−	4−1+	5−	5−	5−
Totals										
E. coli O157:H7 strains containing Stx gene	43	43+	15+	38+	43+	43+	37+6−	40+3−	43+	43−
E. coli O157:H7 strains without Stx gene	19	19−	19−	19−	19−	19−	18−1+	19−	19−	19−

[a] The number positive (+) and the number negative (−) indicate the numbers of strains showing the presence and absence of the gene analyzed, respectively.
[b] That is, EHEC-*hlyA*.

were designed for detection of the left junction (*L4-prrA*) and right junction (*R1-f447*) (Table 1). The left and right junctions of PAI_{CFT071} could not be amplified from strain *E. coli* O157:H7 EDL933. Furthermore, the PCR experiment with *R1-R2* primers did not yield any product in 62 strains of *E. coli* O157:H7, which are linked at the right junction of PAI_{CFT073} (Table 2).

EDL933 and several other *E. coli* O157:H7 isolates were selected for Southern hybridization, including two clinical strains containing Stx gene (strain 223 and 143) and one animal isolate without Stx gene (PC02). The chromosomal DNA of tested strains was extracted by lysozyme-sodium dodecyl sulfate-proteinase K method, which were further purified by the phenol and chloroform extraction method. It was digested with restriction enzyme *Eco*RI and separated on a 0.9% agarose gel. The digested genomic DNA fragments were transferred from the gel to Zeta-Probe BT blotting membranes (Bio-Rad Laboratories, Richmond, Calif.). The PCR products (*prrA, modD, yc73*, and *fepC*) of *E. coli* CFT073, obtained from agarose gels by QIAquick Gel Extraction kit (Gene Company Limited, Beijing, China), were used as probes in the hybridization assays. Digoxigenin labeling of the probes and hybridization were performed with a DNA labeling and detection kit (Promega, Beijing, China). After prehybridization at 68°C for 2 h and the addition of a heat-denatured probe, the blots were incubated overnight (for ca. 16 h) at 68°C in the absence of formamide. The detection was performed according to the manufacturer's instructions. Digoxigenin labeling of PCR fragments of the *prrA* and *modD* gene were used as the probes. Southern hybridization was performed with a DNA labeling and detection kit (Boehringer, Mannheim, Germany). One DNA fragment of *E. coli* O157:H7 EDL933 and other isolates containing the Stx gene hybridized with the probes, of which the molecular size was identical to that of UPEC CFT073 in our condition (Fig. 1). No positive signal was observed for the animal isolate without the Stx gene, as well as for *E. coli* HB101. Hybridization with probes of *yc73* and *fepC* gave the same results (data not shown). These results thus revealed that not all of the pathogenicity-associated island PAI_{CFT073} is present in *E. coli* O157:H7 containing Stx gene, an idea supported by the recently published genome sequence data of *E. coli* O157:H7 EDL933 (16).

A rapid preliminary analysis suggested a co-occurrence between the *prrA-modD-yc73-fepC* gene cluster of PAI_{CFT073} and Stx gene in the *E. coli* O157:H7 strains. Therefore, we carried out a detailed screening to verify this correlation. We analyzed a total of 62 *E. coli* O157:H7 isolates, including 4 isolated from the diarrheal patients in the United States (11), 6 isolated from diarrheal patients in Japan (22), and 52 isolated in China (23–25). All of the isolates were reconfirmed in our laboratory by serological methods for O157 and H7 antigens, as well as by PCR methods for genes of Shiga toxin 1 (*Stx1*), Shiga toxin 2 (*stx2*), EHEC attachment and effacing (*eae*), and hemolysin (EHEC-*hlyA*) (3, 20). The results are shown in Table 2. In 43 *E. coli* O157:H7 strains containing the Stx gene, the frequencies of the detection of the *prrA, modD, yc73*, or *fepC* were 100, 86, 93, and 100%, respectively. In remarkable contrast, none of the *prrA, yc73*, and *fepC* genes was detected in the 19 *E. coli* O157:H7 strains without the Stx gene, and the *modD* gene was detected only in one of them. In 43 strains of *E. coli* O157:H7 containing Stx gene, 37 displayed *prrA-modD-yc73-fepC*-positive PCR pattern, 5 strains were *prrA-yc73-fepC* positive, 2 strains were *prrA-modD-fepC* positive, and one strain showed a *prrA-fepC*-positive PCR pattern.

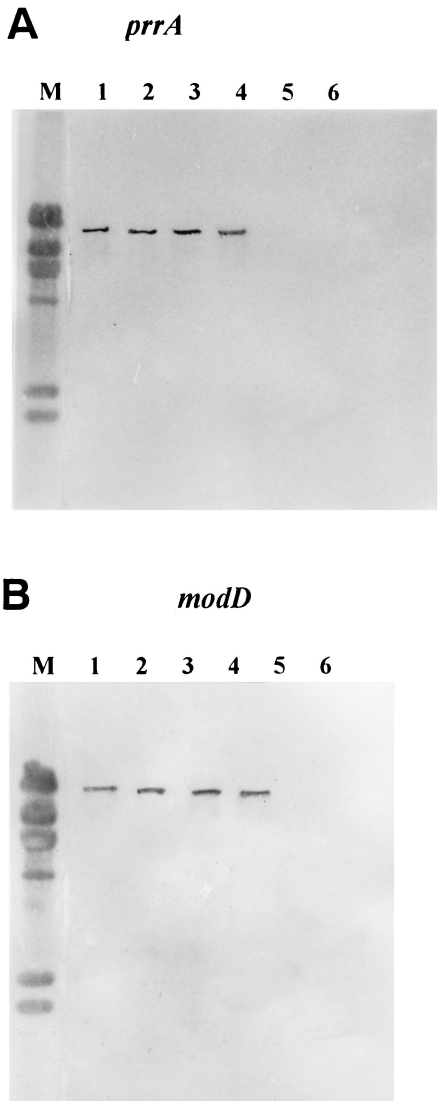

FIG. 1. Southern hybridization profile of the *Eco*RI-digested chromosomal DNA with *prrA* and *modD* probes. Lanes: M, molecular standard (DNA digested with *Hin*dIII); 1, *E. coli* CFT073 (positive control); 2, *E. coli* O157:H7 EDL933; 3, *E. coli* O157:H7 223; 4, *E. coli* O157:H7 143; 5, *E. coli* O157:H7 PC02 (animal isolate without Stx gene); 6, *E. coli* HB101 (negative control). (A) Hybridization with *prrA* probe. (B) Hybridization with *modD* probe.

In order to confirm the PCR results, Southern blots was conducted for nine strains containing Stx gene with various PCR patterns and three *E. coli* O157:H7 strains without the Stx gene. UPEC CFT073 and *E. coli* HB101 were used as positive and negative controls, respectively. The purified chromosome DNA was blotted on a nitrocellulose filter. The PCR products (*prrA, modD, yc73*, and *fepC*) of *E. coli* CFT073, obtained from agarose gels, were used as probes in the hybridization assays. All *E. coli* O157:H7 strains containing the Stx gene tested hybridized with probes of *prrA, modD, yc73*, and *fepC*, regard-

TABLE 3. Confirmation of PCR results by DNA hybridization[a]

Source	Total no. of strains	No. of strains										
		stx	*prrA*		*modD*		*yc73*		*fepC*		*R1-R2*	
			PCR	H	PCR	H	PCR	H	PCR	H	PCR	H
Patient	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3−	3−
Chick	1	1+	1+	1+	1+	1+	1+	1+	1+	1+	1−	1−
Patient	1	1+	1+	1+	1−	1+	1+	1+	1+	1+	1−	1−
Pig	1	1+	1+	1+	1−	1+	1+	1+	1+	1+	1−	1−
Goat	1	1+	1+	1+	1−	1+	1+	1+	1+	1+	1−	
Patient	1	1+	1+	1+	1+	1+	1−	1+	1+	1+	1−	1−
Patient	1	1+	1+	1+	1−	1+	1−	1+	1+	1+	1−	1−
Pig	2	2−	2−	2−	2−	2−	2−	2−	2−	2−	2−	2−
Goat	2	2−	2−	2−	1−1+	2−	2−	2−	2−	2−	2−	2−
E. coli HB101	1	1−	1−	1−	1−	1−	1−	1−	1−	1−	1−	1−
UPEC CFT073	1	1−	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+

[a] PCR and Southern hybridization (H) results are compared. The number positive (+) and the number negative (−) indicate the numbers of strains showing the presence and absence of the gene analyzed, respectively.

less of the PCR patterns. Among these, three were PCR negative for *modD* genes, and two were PCR negative for *yc73* or for *modD-yc73* genes, respectively. These probes did not hybridize with the chromosome of the negative control HB101 under the conditions used. It seems that all of the *E. coli* O157:H7 strains containing the Stx gene have a *prrA-modD-yc73-fepC* gene cluster, and the negative PCR results might be due to the variation in primer sequences of the targeted genes (Table 3). The *E. coli* O157:H7 strain without the Stx gene that yielded *modD* PCR product was failed to hybridize with *modD* DNA probe. It is reasonable to assume that the primers for *modD* gene may yield a false result on some occasions. Therefore, these results demonstrate for the first time a strict correlation between the presence of the *prrA-modD-yc73-fepC* gene cluster in *E. coli* O157:H7 strains containing Stx gene(s). It should be noted that one strain in 43 *E. coli* O157:H7 isolates containing Stx gene had no *eae* gene detected.

In order to assess the similarity in the DNA sequence of the *prrA-modD-yc73-fepC* genes between UPEC CFT073 and *E. coli* O157:H7 EDL933, DNA fragments of the *prrA, modD, yc73*, and *fepC* were amplified by PCR using the chromosomal DNA UPEC CFT073 as a template and extracted from agarose gels by using the QIAquick Gel Extraction Kit and sequenced in both directions by using the *Taq* Dye-Deoxy-Cycle-Sequencing Kit and 373A DNA sequencer (Applied Biosystems, Foster City, Calif.). The sequences obtained were aligned with PAI_{CFT073} sequences published by Guyer et al. with BLAST software (5). The DNA sequences of 479 bp (*prrA*) and 437 bp (*yc73*) of *E. coli* O157:H7 EDL933 were identical to that of UPEC CFT071. In 347 and 378 bp of the *fepC* and *modD* sequences, only two nucleotide mismatches were observed.

The PAI_{CFT073} seems to be atypical. Guyer et al. stated that at the approximately 7 kb downstream of the left junction of PAI_{CFT073}, following the *prrA-modD-yc73-fepC* gene cluster, there are 8-kb sequences carrying six ORFs, which are identical to that found in the *E. coli* K-12 genome (5). The virulent hemolysin gene cluster *hlyCABD* follows this block. The segmentation is obvious with respect to unique PAI_{CFT073} sequences and sequences of *E. coli* K-12 origin. It is reasonable to believe that the *prrA-modD-yc73-fepC* gene cluster, rather than PAI_{CFT073}, is present in *E. coli* O157:H7. Moreover, the *prrA-modD-yc73-fepC* gene cluster might not be a necessary part of PAI_{CFT073} (5). Recently, Tarr et al. identified a tellurite resistance- and adherence-conferring island in *E. coli* O157:H7, in which the 99% DNA sequences of the gene *iha* are identical to *R4* of PAI_{CFT073}, a putative exogenous ferric siderophore receptor (21). In regard to iron uptake, the tellurite resistance- and adherence-conferring island might have some relationship with the *prrA-modD-yc73-fepC* gene cluster in *E. coli* O157:H7 (21).

It seems that the *prrA-modD-yc73-fepC* gene cluster is linked with Shiga toxin gene, so that, it is probably crucial for virulence of *E. coli* O157:H7. Gyer et al. suggested that the *prrA-modD-yc73-fepC* gene cluster represent a TonB-dependent iron uptake system (5). It was suspected that the TonB system is necessary for all gram-negative organisms that dwell in the presence of oxygen. Indeed, the genes encoding homolog to *E. coli* TonB have been cloned and sequenced from *Salmonella enterica* serovar Typhimurium, *Klebsiella pneumoniae, Enterobacter aerogenes, Serratia marcescens, Yersinia enterocolitica, H. influenzae, Pseudomonas putida*, and others. TonB does play a role in addition to heme- and siderophore-mediated iron acquisition in vivo, and this function is related to the bacterial virulence, such as the intercellular spread of *Shigella dysenteriae*, abilities to produce invasive disease in an animal model of *H. influenzae*, and the requirement for mouse virulence of *Y. enterocolitica* (6, 7, 17). It has been demonstrated that the Shiga toxins of *E. coli* O157:H7 are also iron regulated (1, 2, 19). Calderwood and Mekalanos reported that the Shiga toxin operon was negatively regulated by *fur* gene product. In the DNA region between the −35 and −10 boxes of Shiga toxin, the 21-bp dyad repeat may represent an operator binding site for Fur protein in the presence of iron (1). The *fepC* gene has been known to encode ferric enterobactin transport ATP-binding protein (5). Payne and his colleagues have identified an iron transport system in *E. coli* O157:H7 strain EDL933. It has been known that *E. coli* O157:H7 can synthesize and transport enterobactin and had a ferric citrate transport system but lack the ability to produce or use aerobactin. It can use heme and hemoglobin, but not transferrin or lactoferrin, as iron sources. The heme utilization gene (*chuA*) encodes a 69-kDa outer membrane protein, for which the homologous one is also

present in *S. dysenteriae* I, a Shiga toxin-producing species (13). However, the role played by the *prrA-modD-yc73-fepC* gene cluster in the virulence of *E. coli* O157:H7 should be clarified experimentally.

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