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Association of the Urease Gene with Enterohemorrhagic *Escherichia coli* Strains Irrespective of Their Serogroups

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Among various diarrheagenic *Escherichia coli* strains from clinical sources, we found that the urease gene was specifically associated with enterohemorrhagic *E. coli* (EHEC) strains irrespective of their serogroups. The results suggest that the urease gene can be a useful genetic marker for the detection of EHEC strains and for the diagnosis of infections caused by EHEC strains in the clinical situation.

In 1996, a large outbreak of food-borne infection due to enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 occurred in Sakai City, Osaka Prefecture, Japan. The outbreak involved more than 6,000 people and resulted in three deaths (11). Recently, Hayashi et al. (7) reported the complete genome sequence of an EHEC O157:H7 strain isolated from the Sakai outbreak (referred to as strain O157 Sakai). The study demonstrated that a 1,460-kb DNA sequence is specifically present in O157 Sakai but is not found in the *E. coli* K-12 genome (2). Most of the sequence consisted of prophage genomes or regions with prophage-like features (designated Sakai prophage-like elements [SpLEs]) (7). Among the SpLEs, the largest one, SpLE1 (ca. 86 kb long), possesses several genes potentially related to the virulence of the organism. Here we describe the results of our investigation on the distribution of the genes identified on SpLE1 among various diarrheagenic *E. coli* strains.

Strain O157 Sakai (strain RIMD0509952) (7) was used as the standard strain in the present study. The strains examined in the present study consisted 55 diarrheagenic *E. coli* strains including 22 strains of EHEC, 12 strains of enterotoxigenic *E. coli* (ETEC), 8 strains of enteropathogenic *E. coli* (EPEC), 3 strains of enteroaggregative *E. coli* (EAggEC), and 10 strains of enteroinvasive *E. coli* (EIEC) and 4 other enteropathogenic bacteria including 2 strains of *Shigella* spp. (*Shigella flexneri* and *S. dysenteriae*) and 2 strains of *Salmonella* spp. (*Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium). All strains were from our laboratory collection, and all strains except one EHEC strain were clinical isolates, the one exception was derived from a calf. *E. coli* JM109 (13) was also included in the examination as a negative control strain.

All DNA probes used in the study were prepared by PCR amplification. The sequences of the oligonucleotides used as PCR primers are described in Table 1. PCR was performed in a reaction mixture with a total volume of 50 μl. The reaction mixture contained the following components: 0.5 μg of genomic DNA which was extracted from O157 Sakai by a standard method (12), 5 μl of 10× PCR buffer, 4 μl of a deoxynucleoside triphosphate mixture (containing dATP, dCTP, dTTP, and dGTP at concentrations of 10 mM each), 20 pmol of each primer, and 2.5 U of *Taq* DNA polymerase (Wako Pure Chemical Industry Ltd., Osaka, Japan), with the volume completed to 50 μl with distilled water. PCR conditions were as follows: after 3 min of denaturation at 94°C, a cycle of denaturation at 94°C for 1 min, annealing at the optimum temperature for 1 min (Table 1), and extension at 72°C for 1 min was repeated 30 times. The PCR product was separated on a 2% agarose gel and was extracted from the agarose gel with an QIAEXII gel extraction kit (QIAGEN, Hilden, Germany). The DNA probes were labeled with the PCR DIG Probe Synthesis kit, and the hybridized DNAs were detected with alkaline phosphatase-labeled anti-digoxigenin monoclonal antibody (Roche, Indianapolis, Ind.). Hybridization was carried out at 42°C under high-stringency conditions (50% concentration of formamide in hybridization solution) and with washing at 55°C.

SpLE1 encodes 111 open reading frames (ORFs) (>150 bp) ; several ORFs likely encode proteins potentially related to bacterial pathogenesis such as TraT, Iha, AIDA-1, and urease (1, 6, 8–10). We selected seven ORFs found on SpLE1 and examined their distributions among various types of diarrheagenic *E. coli* strains, *Shigella* spp., and *Salmonella* spp. by colony hybridization (Table 2 and Fig. 1).

A significant portion of the EHEC strains reacted with all seven DNA probes (13 of 15 serogroup O157 strains and 3 of 4 serogroup O26 strains), suggesting that SpLE1 or SpLE1-like elements may be widely distributed in EHEC strains, in particular, in serotype O157. In contrast, other types of diarrheagenic *E. coli* and *Shigella* spp. reacted with only some of the DNA probes, if any (Table 2). Among the four probes which reacted with all EHEC strains (probes 1, 3, 5, and 7), probe 3 reacted solely with EHEC strains and not with other *E. coli* strains, while the other probes reacted with some other types of pathogenic *E. coli* strains and *Shigella* spp. The sequence of probe 3 corresponds to the sequence of an internal part of the *ureC* gene, and thus, this finding suggests that the urease

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TABLE 1. Primers used in the present study

Primer designation	Target[a]	Similarity[b]	Probe no.[c]	Sequence	Product size (bp)	Annealing temp (°C)
1299-1 1299-2	ECs1299	Integrase	1	5′-TAAGGCAGTGGTTATCGACG-3′ (20-mer) 5′-ATGCCTGCATCATCGGTACA-3′ (20-mer)	801	63
1312-1 1312-2	ECs1312	TraT	2	5′-GGAATTCTTGAGATTTTCTACAATCG-3′ (26-mer) 5′-GTCGACTCAGAGTATATTGGCGATT-3′ (25-mer)	759	50
1324-1 1324-2	ECs1324	UreC	3	5′-TCTAACGCCACAACCTGTAC-3′ (20-mer) 5′-GAGGAAGGCAGAATATTGGG-3′ (20-mer)	397	60
1360-1 1360-2	ECs1360	Iha	4	5′-ATGATAACCGGGATGGGCAA-3′ (20-mer) 5′-ATGATGCCACCTCTTCGGTG-3′ (20-mer)	964	58
1391-1 1391-2	ECs1391	BfpM	5	5′-GAACAGGGAAATTCAGCAGC-3′ (20-mer) 5′-ATCGACGATTGCTGGAAAGG-3′ (20-mer)	475	60
1396-1 1396-2	ECs1396	Fluffing protein (AIDA-1)	6	5′-ACTGGTTACCAGTACTGCTG-3′ (20-mer) 5′-ACCAGTCTTCATCGCTGTCA-3′ (20-mer)	883	60
1409-1 1409-2	ECs1409	L0010	7	5′-ATATCACAATCTCCCGTCCG-3′ (20-mer) 5′-AGTCTGTCAACCAGTTCTGG-3′ (20-mer)	790	63

[a] The names of the ORFs are according to Hayashi et al. (7).
[b] Protein in GenBank database showing highest degree of similarity.
[c] Probe number described in Fig. 1.

operon may be specifically and ubiquitously distributed in EHEC strains, at least in serogroups O157, O26, and O111.

Since it was demonstrated that all EHEC strains tested possess the *ureC* gene, irrespective of their serogroups, we examined the urease activities of these EHEC strains including O157 Sakai. Among 23 EHEC strains tested, only 1 strain (a serogroup O157 strain) showed urease activity when urea agar base (Becton Dickinson, Sparks, Md.) was used. These results suggest that, at least under the conditions used in the present study, urease production could not be detected in most of the EHEC strains tested, despite their possession of the *ureC* gene.

It is of particular importance that probe 3, whose sequence corresponds to the sequence of an internal part of *ureC*, reacted with all EHEC strains tested but none of the other types of diarrheagenic *E. coli* strains. This suggests that the urease operon is uniquely present in EHEC strains, irrespective of the serogroup. Although the production of urease was not detected in most EHEC strains examined in the present study, the urease gene will be a useful marker for differentiation of EHEC strains from other diarrheagenic *E. coli* strains.

E. coli infections are not limited to gastroenteritis. Therefore, it is noteworthy that uropathogenic *E. coli* (UPEC) was reported to contain the urease operon (3, 4). Although the full sequence of the UPEC *ureC* gene is not available, phylogenetic analysis of UreA, UreB, and UreG shows that the urease genes of EHEC strains exhibit the highest degree of similarity to those of *Klebsiella aerogenes*, while the urease genes of UPEC are most similar to those of *Proteus mirabilis* (data not shown). Furthermore, the gene organizations of the urease operons were different from each other in EHEC and UPEC strains (5). These data suggest that an appropriate primer set can differentiate the EHEC *ureC* gene from the UPEC *ureC* gene. We are now examining this possibility by analyzing a larger number of clinical *E. coli* isolates from patients with both gastrointestinal and urinary tract infections.

TABLE 2. Distributions of ORFs located on SpLE1 in diarrheagenic *E. coli* strains and enteropathogens

Type	Serogroup	No. of strains	No. (%) of strains positive with the following ORF probe:						
			1	2	3	4	5	6	7
EHEC	O157	15	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	13 (86.7)	15 (100)
EHEC	O26	4	4 (100)	4 (100)	4 (100)	3 (75.0)	4 (100)	3 (75.0)	4 (100)
EHEC	O111	3	3 (100)	2 (66.7)	3 (100)	2 (66.7)	3 (100)	0	3 (100)
ETEC		12	0	0	0	0	0	2 (16.7)	1 (8.3)
EPEC		8	0	1 (12.5)	0	2 (25.0)	7 (87.5)	6 (75.0)	0
EIEC		10	0	8 (80.0)	0	7 (70.0)	9 (90.0)	1 (10.0)	4 (40.0)
EAggEC		3	1 (33.3)	1 (33.3)	0	3 (100)	0	1 (33.3)	3 (100)
Shigella[a]		2	0	2 (100)	0	0	2 (100)	1 (50.0)	1 (50.0)
Salmonella[b]		2	0	0	0	0	0	0	0
E. coli JM109			0	0	0	0	0	0	0

[a] *S. flexneri* and *S. dysenteriae*.
[b] *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium.

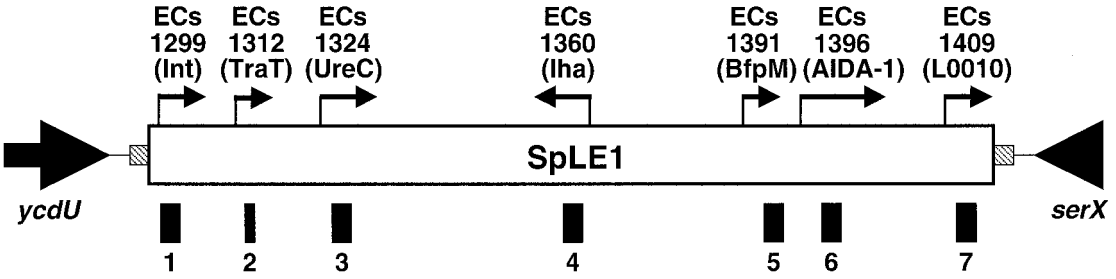

FIG. 1. Locations of the DNA probes on SpLE1. Arrows and shaded boxes indicate the direction of transcription and the direct repeated sequences, respectively. The black boxes below SpLE1 represent the locations of the DNA probes.

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