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An *Escherichia coli* Reference Collection Group B2- and Uropathogen-Associated Polymorphism in the *rpoS-mutS* Region of the *E. coli* Chromosome

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Chromosomal DNAs of enterohemorrhagic, uropathogenic, and laboratory attenuated *Escherichia coli* strains differ in the *rpoS-mutS* region. Many uropathogens lack a deletion and an insertion characteristic of enterohemorrhagic strains. At the same chromosomal position, they harbor a 2.1-kb insertion of unknown origin with a base composition suggestive of horizontal gene transfer. Unlike virulence determinants associated with urinary tract infection and/or neonatal meningitis (*pap* or *prs*, *sfa*, *kps*, and *hly*), the 2.1-kb insertion is shared by all group B2 strains of the *E. coli* Reference Collection.

Genomic sequencing offers unprecedented opportunities for the identification of genetic polymorphisms related to bacterial evolution and virulence. The complete nucleotide sequence of *Escherichia coli* MG1655 (4), a representative laboratory-attenuated *E. coli* K-12 strain, provides a foundation for studies of the evolution and virulence of *E. coli* strains associated with diverse pathologies. The expanding list of *E. coli* virotypes includes diverse diarrheagenic organisms (labeled enterotoxigenic, enteropathogenic, enterohemorrhagic, enteroaggregative, enteroinvasive, and diffusely adherent) (28) as well as isolates associated with extraintestinal diseases, including neonatal meningitis (7) and urinary tract infections (UTIs) (including bacteriuria, cystitis, and pyelonephritis) (11). By complementing phenotypic analysis and multilocus enzyme electrophoresis, sequence comparisons are now providing profound insights into the pathogenesis and evolution of *E. coli* (5, 12, 19, 23–25, 28, 29, 37, 39–42). These and other studies (13, 15) reveal that the chromosomal DNA sequences of modern organisms reflect both their clonal origins and horizontal gene transfer.

A uropathogen-associated, *rpoS*-proximal DNA polymorphism in *E. coli*. Recently, LeClerc et al. (21) reported that, in comparison to *E. coli* MG1655, *E. coli* O157:H7, related enterohemorrhagic *E. coli* strains, and *Shigella dysenteriae* lack 6.1 kb of chromosomal DNA and harbor a 2.9-kb DNA insertion in the *rpoS-mutS* intergenic region (61.5 to 61.7 map units) (Fig. 1). While deleting the *rpoS* locus from uropathogenic *E. coli* strains during a study of osmoregulation and virulence (9, 10), we discovered a different polymorphism at the same location. *E. coli* strain CFT073, a highly virulent isolate from a patient with pyelonephritis (27), appears to retain the full *rpoS-mutS* intergenic sequence characteristic of *E. coli* MG1655 (Fig. 1). In addition, a 2.1-kb DNA insert replaces the 2.9-kb sequence identified by LeClerc et al. (21). This insert was initially detected when attempts to PCR amplify *rpoS* failed to produce a DNA fragment of the expected size (primers A and G) (Fig. 2). Genomic DNA from *E. coli* CFT073 was prepared as follows (36). Bacteria harvested from a 1-ml overnight culture in Luria-Bertani medium (26) were washed once with 1 ml of saline (0.85% [wt/vol] NaCl), resuspended in 0.5 ml of distilled water, boiled for 10 min, and chilled on ice. Debris was removed by centrifugation, and the relevant sequences were PCR amplified with 5 μl of the resulting extract as a template (8). The insert sequence was determined by GenAlyTiC (University of Guelph, Guelph, Ontario). Additional primers were created as required to complete this 2.1-kb sequence (Fig. 2). The inserted DNA occurs at exactly the same location as that present in *E. coli* O157:H7 but differs in both size and sequence (the insert sequences are not related). It has a base composition of 40% G+C, a value much lower than the average for *E. coli* K-12 (50%) and for the immediately flanking sequences (52%). The insert may therefore have arrived in *E. coli* by horizontal gene transfer (29).

To determine the distribution of the inserted sequence, we applied PCR analysis to chromosomal DNA isolated from diverse clinical *E. coli* isolates (Table 1). DNA was prepared (36), and PCR was performed (8) using the primers listed in Table 1 and Fig. 2B (two pairs of primers per PCR). Production of a 301-bp amplicon during test 1 (primers C and D) indicated the presence of the inserted sequence. Production of 579- (primers E and F) and 483-bp (primers A and B) amplicons during test 2 indicated its location in the *rpoS-mutS* region. PCR amplification of the housekeeping locus *putP*, located at 23.3 map units, provided a positive control for the quality of the DNA templates. A DNA insert similar to that discovered in pyelonephritis isolate CFT073 was present in a majority of UTI isolates, including bacteria isolated from patients with uncomplicated pyelonephritis (7 of 7) or cystitis (8 of 12) and unspecified UTIs (4 of 4). It was present in approximately one-half of the tested isolates from patients with catheter-associated infections (3 of 7), and it was uncommon among bacteria isolated from patients with hemorrhagic colitis (0 of 5) or infantile diarrhea (2 of 21). When present, it was located in the *rpoS-mutS* region (Table 1).

No 301-bp PCR product (internal to the DNA insert) was observed when PCR test 1 was applied with chromosomal DNA from *Salmonella enterica* serovar Typhimurium, *Klebsiella oxytoca*, *Pseudomonas putida*, *Pseudomonas paucimobilis*, *Vibrio anguillarum*, *Yersinia ruckeri*, *Erwinia carotovora*, *Hafnia alvei*, *Enterobacter cloacae*, or *S. dysenteriae* as a template. Like that from some *E. coli* isolates listed in Table 1, chromosomal DNA from *S. dysenteriae* supported DNA amplification with PCR test 2, but the resulting pattern of DNA fragments differed from that observed with *E. coli* CFT073 DNA as a tem-

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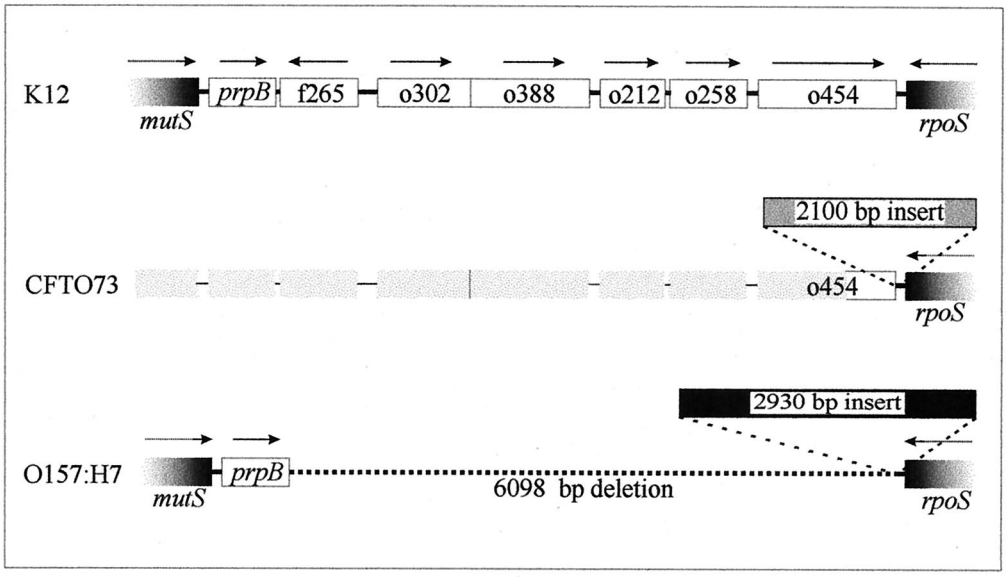

FIG. 1. Comparison of the *rpoS-mutS* intergenic regions of *E. coli* K-12 (laboratory) (4), *E. coli* CFT073 (pyelonephritis) (this study), and *E. coli* O157:H7 (hemorrhagic colitis) (21). *E. coli* CFT073 lacks the 6.1-kb DNA deletion in the *rpoS-mutS* intergenic region that is characteristic of enterohemorrhagic strains (e.g., O157:H7). However the presence of the full 6.1-kb *rpoS-mutS* intergenic sequence found in *E. coli* MG1655 has not been verified for *E. coli* CFT073.

plate. Thus, the DNA insert observed in *E. coli* CFT073 was different from that found in *E. coli* O157:H7 and *S. dysenteriae* type 1 (21). It was more common among UTI isolates than among the other clinical *E. coli* isolates included in this study, and it was not detected by PCR amplification in an array of other organisms.

Analysis of the DNA sequence inserted in *E. coli* CFT073 revealed two open reading frames (ORFs) encoding proteins greater than 10 kDa in molecular mass for which similar sequences could be found (Fig. 2 and Table 2). ORF183 showed 26% identity to WrbA, a flavodoxinlike protein that is expressed by *E. coli* K-12 during stationary phase and whose

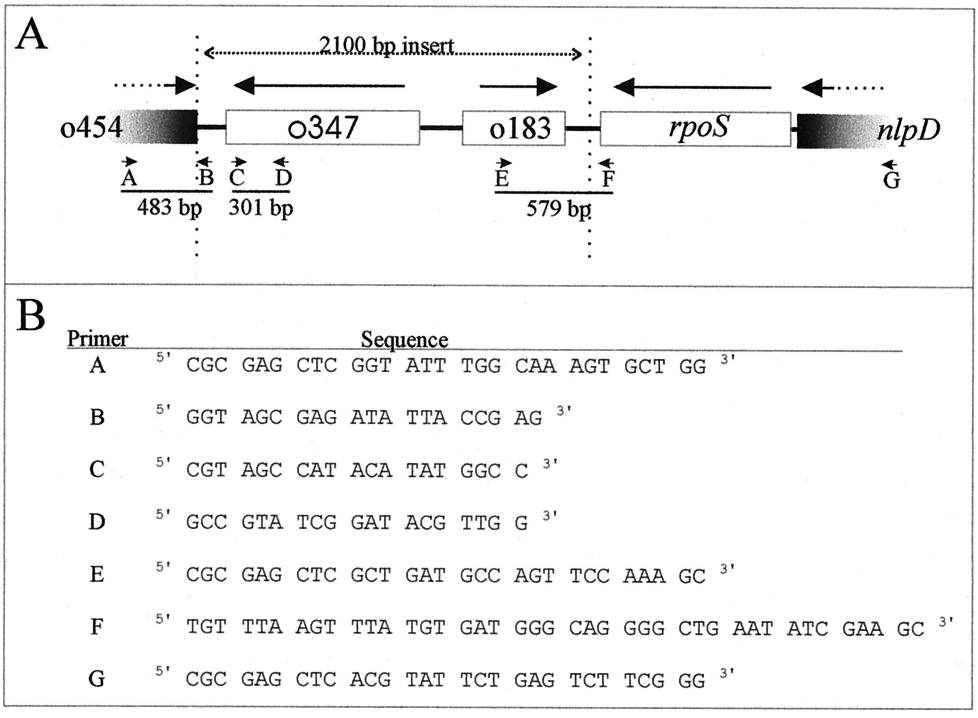

FIG. 2. The *rpoS-mutS* intergenic region of *E. coli* CFT073 (accession no. AF270497) and its detection by PCR amplification. (A) Physical map, showing positions of ORFs (Table 2) and locations of DNA fragments amplified by PCR (Tables 1 and 3). (B) PCR primers used to perform the multiplex PCR analyses reported in Tables 1 and 3.

TABLE 1. Distribution of the inserted sequence among clinical *E. coli* strains

Strain or isolate(s)	Origin	PCR test 1[a]		PCR test 2[b]		Source or reference
		putP (202 bp)	Internal fragment (301 bp)	*rpoS* border (579 bp)	*o454* border (483 bp)	
ATCC25992	Clinical	+	+	+	+	G. Reid
BB593:2b	Fecal (child)	+	+	+	+	G. Reid
Co1	Fecal (non-UTI)	+	−	−	−	G. Reid (35)
SM47[c]	Neonatal meningitis	+	−	−	−	G. Reid
2 Isolates[d]	Hemorrhagic colitis	+	−	−	−	C. L. Gyles
3 Isolates	Hemorrhagic colitis	+	−	NT	NT	C. L. Gyles
3 Isolates[d]	Infantile diarrhea	+	−	−	−	C. L. Gyles
16 Isolates	Infantile diarrhea	+	−	NT	NT	C. L. Gyles
2 Isolates	Infantile diarrhea	+	+	+	+	C. L. Gyles
1 Isolate[c]	Catheter	+	−	−	−	G. Reid
3 Isolates[d]	Catheter	+	−	−	−	G. Reid
3 Isolates	Catheter	+	+	+	+	G. Reid
431	Bacteriuria	+	+	+	+	G. Reid (35)
950	UTI (bacteremia)	+	+	+	+	G. Reid (38)
2239	UTI	+	+	+	+	G. Reid
C1212	UTI	+	+	+	+	G. Reid (31)
C1214	UTI	+	+	+	+	G. Reid (31)
2 Isolates	Cystitis	+	−	−	−	G. Reid
2 Isolates[d]	Cystitis	+	−	−	−	G. Reid (33, 34)
8 Isolates	Cystitis	+	+	+	+	G. Reid (33, 34)
CFT073	Acute pyelonephritis	+	+	+	+	H. Mobley (27)
HU734	Acute pyelonephritis	+	+	+	+	G. Reid (16, 17)
5 Isolates	Pyelonephritis	+	+	+	+	G. Reid

[a] Multiplex PCR test 1 was performed with *putP* primers plus primers C and D (Fig. 2). The *putP* primers were putP1, 5′-GGTTGCGTGTGCATACCGA-3′ (bp 287 to 305 of *putP*), and putP2, 5′-GCCGTTTCGTAGCTCATGC-3′ (bp 469 to 488 of *putP*). +, DNA fragments of the indicated sizes were obtained; −, PCR yielded no DNA fragment.

[b] Multiplex PCR test 2 was performed using primers A, B, E, and F (Fig. 2). +, DNA fragments of the indicated sizes were obtained; −, PCR yielded no DNA fragment; NT, PCR test 2 was not performed.

[c] Multiplex PCR test 2 yielded a single 526-bp DNA fragment (characteristic of *E. coli* K-12).

[d] Multiplex PCR test 2 yielded a DNA fragment pattern unlike that of *E. coli* CFT073 or *E. coli* MC4100.

sequence homologues have been found in bacteria, yeast, and plants (14). ORF347 showed comparable, limited levels of similarity to enzymes implicated in antibiotic hydrolysis (1) and synthesis (2). Though the base composition of the DNA encoding ORF183 was similar to that of *E. coli* K-12 (49%), the base composition of the DNA encoding ORF347 was much lower (39%), suggesting that they may differ in origin. No sequence similarity was sufficiently high to suggest the recent transfer of the entire 2.1-kb sequence or its subfragments from another organism.

The inserted sequence is present in all members of the ECOR group B2. The *E. coli* Reference (ECOR) Collection, a set of *E. coli* strains isolated from diverse hosts and geographic locations, was designed to represent the variation and genetic structure of *E. coli* (30). Studies of housekeeping loci, applied to these strains and others, clearly define the clonal nature of natural *E. coli* populations (18, 40). We explored the evolutionary origin of the 2.1-kb *rpoS*-proximal inserted sequence by examining its occurrence among the 72 ECOR strains (Table 3). PCR test 1 (Table 1) detected this sequence in ECOR strains EC23 and EC32 and in each of the ECOR group B2 strains. UTI-related virulence determinants, believed to have arrived by horizontal gene transfer, occur at higher frequency within ECOR group B2 than among other ECOR strains (Table 3) (6, 20, 22). The presence of the *rpoS*-proximal 2.1-kb insertion within group B2 members is therefore consistent with its presence in many UTI isolates (Table 1). This insertion is present in all group B2 isolates, whereas few contain all of the tested UTI-linked virulence determinants (*pap*, *prs*, *sfa*, *kps*, and *hly*), each of which varies in chromosome map position among *E. coli* isolates. It is thus likely that the 2.1-kb insertion arrived earlier than these virulence determinants during the evolution of group B2.

Some data suggest that genomic sequences common to group B2 organisms diverge deeply from those of commensal *E. coli* strains in ECOR groups A and B1 and have provided an

TABLE 2. Proteins related to ORFs encoded by the inserted sequence

ORF		Similar sequence					
Name	%G+C	Name	Organism	Function	Size (aa)[a]	%ID[b]	Reference
o183	49	WrbA	*E. coli* K-12	Stationary phase; flavodoxinlike protein	202	26	14
o347	39	IND-1	*Chryseobacterium indologenes*	β-Lactamase precursor	239	27	1
		ORF4	*Streptomyces roseofulvus*	Polyketide antibiotic biosynthesis (cyclase)	314	25	2

[a] aa, amino acids.

[b] %ID, percent identity.

TABLE 3. Properties of ECOR strains harboring the inserted sequence[a]

Strain	ECOR group	Distribution of UTI-associated virulence determinants[b]			
		pap or *prs*	*sfa*	*kps*	*hly*
EC23	A	−	−	−	−
EC32	B1	−	−	−	−
EC51	B2	+	+	+	+
EC52	B2	+	+	+	+
EC54	B2	−	+	+	+
EC56	B2	+	−	+	+
EC57	B2	+	+	+	+
EC55	B2	+	−	+	−
EC65	B2	−	+	−	+
EC61	B2	−	−	+	−
EC62	B2	+	−	+	−
EC63	B2	+	+	+	+
EC64	B2	+	+	+	−
EC53	B2	+	+	+	+
EC59	B2	−	−	−	−
EC60	B2	+	+	−	+
EC66	B2	+	+	+	−

[a] Evidence for the presence and location of the *rpoS*-proximal inserted sequence in the ECOR strains was sought by multiplex PCR test 1 with PCR-based detection of *putP* as a control (see the text and Table 1). The listed strains, ordered according to their evolutionary relationships (18), contained both *putP* and the *rpoS*-proximal insertion sequence as indicated by both tests. The other 55 ECOR strains (not listed) contained the former but not the latter sequence.

[b] The mnemonics refer to genetic loci encoding the following virulence determinants: *pap*, pyelonephritis-associated or P pili with class I adhesins; *prs*, pyelonephritis-associated or P pili with class III adhesins; *sfa*, S-fimbrial adhesins; *kps*, type II capsule; *hly*, α-hemolysin (6, 22). +, present; −, absent.

essential context for the evolution of extraintestinal virulence (3, 32). Bingen et al. compared the distribution of ribotypes and virulence markers associated with extraintestinal infections for 69 neonatal meningitis isolates and for the ECOR strains (3). The neonatal meningitis isolates were concentrated in phylogenetic group B2. Though present in all phylogenetic groups, virulence markers linked to neonatal meningitis (including *sfa* or *foc* and *ibe-10*) were also present at the highest frequency in group B2. In contrast, the UTI-associated marker *pap* was present at the highest frequencies in non-B2 neonatal meningitis isolates and in group B2 ECOR strains. The 2.1-kb *rpoS*-proximal DNA insertion present in group B2 ECOR strains and many uropathogens was not detected in the single neonatal meningitis isolate included in this study (Table 1). Given their concentration in group B2, the 2.1-kb sequence may be found within other neonatal meningitis isolates.

Nucleotide sequence accession number. The 2.1-kb insert in the *rpoS-mutS* intergenic region of *E. coli* CFT073 was registered with GenBank under accession no AF270497.

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