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Analysis of the Enterohemorrhagic *Escherichia coli* O157 DNA Region Containing Lambdoid Phage Gene *p* and Shiga-Like Toxin Structural Genes

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In this study, we determined the nucleotide sequence of the *p* gene contained within a 5-kb *Eco*RI restriction fragment cloned from Shiga-like toxin II (SLT-II)-converting phage 933W of *Escherichia coli* O157:H7 strain EDL933. The *p* gene was 702 bp long and had 95.3% sequence similarity to the *p* gene of phage λ. Multiple hybridization patterns were obtained when genomic DNA fragments were hybridized with both *p* and *slt-I*, *slt-II*, or *slt-IIc* sequences. All O157 isolates also possessed an analog of λ gene *p* which was not linked with either *slt-I* or *slt-II*. Restriction fragment length polymorphism comparisons of clinical O157 isolates and derivates undergoing genotype turnover during infection were made, and loss of large DNA fragments that hybridized with *slt-II* and *p* sequences was observed. To further analyze the DNA region containing the *p* and *slt* genes, we amplified fragments by using a PCR with one primer complementary to *p* and the other complementary to either the *slt-I* or the *slt-II* gene. PCR analysis with enterohemorrhagic *E. coli* O157 and non-O157 strains yielded PCR products that varied in size between 5.1 and 7.8 kb. These results suggest that even within O157 isolates, the genomes of SLT-converting phages differ. The methods described here may assist in further investigation of SLT-encoding phages and their role in the epidemiology of infection with enterohemorrhagic *E. coli*.

Enterohemorrhagic *Escherichia coli* (EHEC) O157 causes hemorrhagic colitis and hemolytic-uremic syndrome (HUS) and has been isolated from patients throughout the world. Because EHEC O157 can be transmitted through contaminated food and water, as well as by infected persons, clinical microbiologists are increasingly asked not only to identify but also to type O157 to clarify the chain of infection. The typing methods currently employed include phage typing (1), multilocus enzyme electrophoretic typing (25), Shiga-like toxin (SLT) genotyping (5), plasmid typing (11), random amplified polymorphic DNA fingerprinting (24), and genomic DNA restriction fragment length polymorphism (RFLP) analyses (3, 12).

Molecular methods have demonstrated that O157 isolates have the potential to rapidly change their genotypic composition. This phenomenon, referred to as clonal turnover, can occur within the O157 population of an individual patient and is characterized by the appearance of new clonal genotypes and loss of old clones (8). It is possible that prophages known to be integrated in the O157 chromosome are partly responsible for this phenomenon. Phages that contain the structural genes for SLT-I or SLT-II have been isolated from O157 strains, and their morphology, genome sizes, and RFLP have been characterized (9, 14, 20, 22). No differences in morphology have been reported among SLT-converting phages from *E. coli* O157:H7 and O157:H$^-$ strains (14). Moreover, the O157 phages are similar in genome size (62 to 73 kb) and have highly related RFLP patterns (14, 26). In contrast to the highly conserved RFLP of isolated O157 phages, the genomic DNAs of different O157 isolates have revealed uniquely differing RFLP patterns when probed with a 7.4-kb *Pvu*II fragment from O157 toxin-converting phage 933W (9) or with total λ DNA (12). λ RFLP analysis was recently recommended as a reliable epidemiological tool for discriminating between O157 strains and thus for establishing the relationship between strains involved in different outbreaks (18). The reasons for differences in the observed restriction patterns are unknown.

DNA probes and PCR procedures which allow the detection of DNA fragments containing both phage and *slt* sequences may enhance the interpretation of RFLP patterns and understanding of their heterogeneity. Such techniques could also assist in the identification of epidemiologically linked isolates which differ by their DNA fingerprints. The *p* gene is one gene of interest because of its importance in the replication of lambdoid phages (19); for O26 phage H19, the *p* gene has been shown to be located near the *slt-I* gene by hybridization studies (6).

In this study, we identified and sequenced the *p* gene of SLT-II-converting O157 phage 933W. We then used *p* gene sequences in hybridization experiments to determine the number of *p* genes present in O157 and to study their RFLP and their linkage with distinct *slt* genes. We succeeded in developing a PCR which yielded fragments hybridizing with both *p* and *slt-I* or *slt-II* sequences.

(Part of this work will appear in the Ph.D. thesis of Claudia Janetzki-Mittmann.)

MATERIALS AND METHODS

Bacterial strains and phages. The clinical O157 and non-O157 *E. coli* strains investigated in this study, their origins, and their disease association are listed in Table 1. Pheno- and genotyping of these strains was performed as described previously (17). To establish whether the initial O157 culture had heterogeneous

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TABLE 1. Disease association and *slt* genotypes of clinical *E. coli* isolates investigated in this study

Strain no.[a]	Serotype	*slt* genotype	Disease association or source	Reference or source
47-1/93	O157:H7	*slt-II slt-IIc*	HUS	8
47-2/93	O157:H7	*slt-IIc*	HUS	8
EDL933	O157:H7	*slt-I slt-II*	Meat	CDC[b]
1249/87	O157:H7	*slt-II slt-IIc*	HUS	16
1658/91	O157:H7	*slt-II slt-IIc*	HUS	17
3574/92	O157:H7	*slt-II*	HUS	This study
4821/87	O157:H7	*slt-IIc*	D[c]	16
6578/91	O157:H7	*slt-I*	HUS	17
7513/91	O157:H7	*slt-IIc*	HUS	16
9167/91	O157:H7	*slt-I slt-IIc*	HUS	This study
12-1/92	O157:H$^-$	*slt-II slt-IIc*	D	8
12-2/92	O157:H$^-$	*slt-IIc*	D	8
SF 493/89	O157:H$^-$	*slt-II*	HUS	7
1193/89	O157:H$^-$	*slt-I*	HUS	17
1567-1/93	O157:H$^-$	*slt-II slt-IIc*	D	This study
1567-2/93	O157:H$^-$	*slt-IIc*	D	This study
2785-1/93	O157:H$^-$	*slt-II slt-IIc*	HUS	This study
2785-2/93	O157:H$^-$	*slt-IIc*	HUS	This study
3978/91	O157:H$^-$	*slt-II slt-IIc*	HUS	16
SF 4162/94	O157:H$^-$	*slt-II*	HUS	This study
5159/91	O157:H$^-$	*slt-II*	HUS	17
5291/92	O157:H$^-$	*slt-I slt-IIc*	HUS	16
6537/91	O157:H$^-$	*slt-II slt-IIc*	HUS	17
7576/92	O157:H$^-$	*slt-II*	HUS	17
E-D147	O26:H11	*slt-II*	HUS	15
E-D17	O26:H$^-$	*slt-I*	HUS	15
78/92	O111:H$^-$	*slt-I*	HUS	15
1166/93	O111:H$^-$	*slt-II*	HUS	This study

[a] "-1" or "-2" within a strain designation means that the isolate was from the first or a following stool sample of a single patient; e.g., strains 2785-1/92 and 2785-2/92 are isolates from the same patient. SF, sorbitol fermenting.
[b] CDC, Centers for Disease Control and Prevention, Atlanta, Ga.
[c] D, diarrhea.

SLT genotypes and whether the genotypes of the O157 strains in the follow-up stools differed, SLT genotyping with 8 to 16 colonies from the primary isolation medium and the same number of colonies from a convalescent-phase stool sample obtained 32 days after the onset of diarrhea was performed as previously described (17). In four patient stool samples, the primary isolates (*E. coli* 47-1/93, 12-1/92, 1567-1/93, and 2785-1/93 [Table 1]) had *slt-IIc* and *slt-II*, whereas the isolates (*E. coli* 47-2/93, 12-2/92, 1567-2/93, and 2785-2/93 [Table 1]) obtained from convalescent-phase stool samples possessed only *slt-IIc*. The genotypes of some of these strains have been described before (8, 15–17). *E. coli* DH5α (BRL Life Technologies, Inc., Gaithersburg, Md.) and C600 (2) served as negative controls in the hybridization and PCR studies. *E. coli* C600(H19J) and C600(933W) are laboratory strains lysogenized with O26 phage H19J (21) and O157 phage 933W (22), respectively. Phage 933W was induced by mitomycin C and purified as previously described (10).

Cloning and sequencing of the *p* gene from phage 933W. A 5-kb *Eco*RI fragment of lambdoid phage 933W was cloned into vector pK18 (13). Sequence analysis was performed with the M13/pUC sequencing and reverse sequencing oligonucleotides (Boehringer GmbH, Mannheim, Germany); customized primers were used to analyze the middle part of the DNA fragment. Separation of sequencing products was performed on 7% denaturing polyacrylamide gels in a 373A automatic sequencer (Perkin Elmer-Applied Biosystems GmbH, Weiterstadt, Germany) as previously described (16). Nucleotide sequencing was carried out in triplicate and analyzed with the DNASIS program, version 2.0, from Hitachi Software (San Bruno, Calif.).

Development of a PCR assay and Southern blot hybridization. Digoxigenin-labeled *slt-IB* and *slt-IIB* probes were prepared by PCR with the primer pairs KS7-KS8 (15) and GK5-GK6 (17) (Table 2). To amplify *p* gene sequences of phage λ, we constructed primers DK1 and DK2 (Table 2), which were derived from the published nucleotide sequence of phage λ (19). The PCR conditions used to amplify the *p* gene were the same as those described for *slt-IB* (15). Phage λ DNA was purchased from GIBCO BRL, Eggenstein, Germany, and labeled with the digoxigenin labeling and detection kit (Boehringer GmbH). A digoxigenin-labeled, PCR-generated gene *p* probe was used for hybridization. Genomic DNA of *E. coli* was digested with different restriction endonucleases in accordance with the manufacturer's instructions (GIBCO BRL) and subjected to electrophoresis through a 0.5% agarose gel. The digested DNA fragments were transferred onto nylon membranes (Zeta probe; Bio-Rad, Hercules, Calif.) by capillary blotting. Hybridization was performed with the digoxigenin-labeled *slt-IB*, *slt-IIB*, and *p* gene-specific DNA probes under stringent conditions in accordance with the Boehringer manual. The digoxigenin-labeled oligonucleotide specific for the *slt-IIcB* gene (23) was purchased from Roth GmbH, Karlsruhe, Germany, and used for hybridization as previously described (23).

Amplification of DNA fragments with *slt* and *p* gene-specific primers. Amplification of the chromosomal DNA fragment between the *slt-IB* and *p* genes was done with the primer pair DK1-KS8. Primers DK1 and GK7 (Table 2) were designed to amplify the DNA between genes *slt-IIA* and *p*. PCR was performed in the GeneAmp PCR System 9600 (Perkin Elmer-Applied Biosystems GmbH). Amplification was carried out in a total volume of 50 μl containing each deoxynucleoside triphosphate at 200 mM, 30 pmol of each primer, 5 μl of 10-fold-concentrated polymerase synthesis buffer, and 2.6 U of Expand High Fidelity Polymerase (Boehringer GmbH). Template DNA (500 ng) was denatured at 94°C for 15 s, annealed at 45°C for 30 s, and then extended for 4 min at 68°C. This amplification was carried out for 10 cycles. In the following 20 cycles, the elongation time was 4 min plus a 20-s elongation step for each cycle. A final extension step of 7 min at 72°C was conducted. The PCR products were separated on a 0.8% agarose gel. Gel slices with the appropriate DNA fragment were excised, and the DNA was eluted and purified with the Prep-a-gene kit (Bio-Rad, Munich, Germany). Purified DNA was digested either with *Pvu*II or with *Hin*dIII and separated again on a 0.8% agarose gel. The DNA was transferred from agarose gels to nylon membranes and hybridized with the *p* gene-specific probe as previously described (17). To further confirm the identities of the PCR fragments obtained, the purified fragments were subjected to sequence analysis by the *Taq* cycle sequencing method described above.

Nucleotide sequence accession number. The nucleotide sequence of the *p* gene of phage 933W was submitted to the EMBL data library and assigned accession number X84263.

RESULTS

Cloning and sequence analysis of gene *p* of O157 phage 933W. A 402-bp probe complementary to sequences of the *p* gene from phage λ was used to hybridize DNAs from phages 933W and λ (Fig. 1A and B). A 5-kb *Eco*RI DNA fragment of phage 933W demonstrating a hybridization signal with the *p* gene probe (Fig. 1A, lane 1) was cloned into vector pK18; a 400-bp segment from the 3′ end and a 1,500-bp segment from the 5′ end were sequenced. In the latter sequence, we detected a 702-bp open reading frame that revealed 95.3% nucleotide similarity to the *p* gene of λ.

RFLP of *p* genes in *E. coli* O157 strains. To determine the *p* gene RFLPs in *E. coli* O157 strains, genomic DNAs of 14 O157

TABLE 2. Nucleotide sequences of primers used for PCR

Primer	Nucleotide sequence	Gene (corresponding nucleotides)	Reference
GK5	5′-ATGAAGAAGATGTTTATGGCG-3′	*slt-IIB* or *slt-IIcB* (bp 1–21)	17
GK6	5′-TCAGTCATTATTAAACTGCAC-3′	*slt-IIB* or *slt-IIcB* (bp 270–250)	17
GK7	5′-CGAAGAGACATAACTTTG-3′	*slt-IIA* or *slt-IIcA* (bp 114–97)	This study
KS7	5′-CCCGGATCCATGAAAAAAACATTATTAATAGC-3′[a]	*slt-IB* (bp 1–23)	15
KS8	5′-CCCGAATTCAGCTATTCTGAGTCAACG-3′[a]	*slt-IB* (bp 282–265)	15
DK1	5′-ACATCGCCGCACAGATGG-3′	*p* (bp 39589–39605 in phage λ sequence)	This study
DK2	5′-GCTTCCGGCAATACTCGT-3′	*p* (bp 39990–39972 in phage λ sequence)	This study

[a] Nucleotides underlined are restriction sites and 5′ extensions which are not from the *slt-IB* sequence.

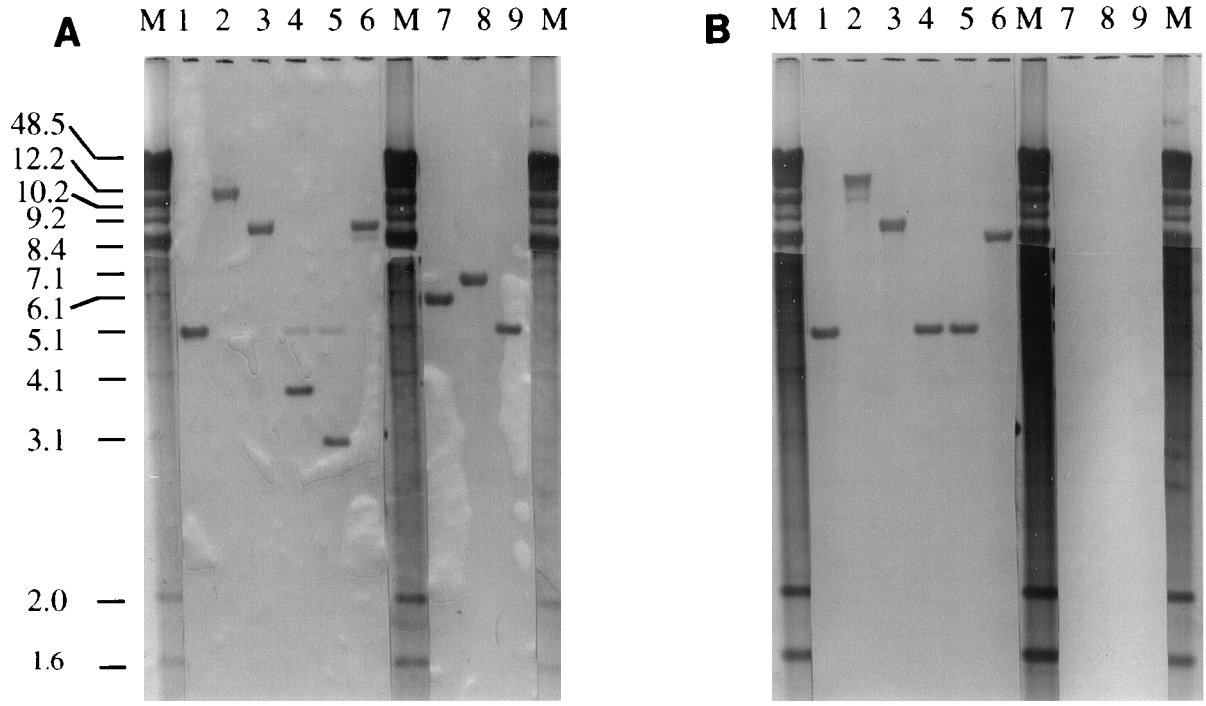

FIG. 1. Southern blot analysis of digested DNAs from phages 933W (A) and λ (B) hybridized with a PCR-generated, digoxigenin-labeled *p* (lanes 1 to 6) or *slt-IIB* (lanes 7 to 9) probe. DNA was digested with the enzymes *Eco*RI (lane 1), *Hin*dIII (lane 2), *Sal*I (lane 3), *Eco*RI-*Hin*dIII (lane 4), *Eco*RI-*Sal*I (lane 5), *Sal*I-*Hin*dIII (lane 6), *Eco*RI (lane 7), *Hin*dIII (lane 8), and *Eco*RI-*Hin*dIII (lane 9). Lane M contained a cocktail of two molecular size markers: a 1-kb DNA ladder and a high-molecular-weight marker (GIBCO BRL). The values to the left are molecular sizes in kilobase pairs.

strains belonging to six *slt* genotypes and control strains were digested with *Eco*RI, *Sal*I, and *Hin*dIII and subsequently hybridized with the *p* gene probe. No hybridization occurred when DNAs of the *E. coli* laboratory strains were subjected to Southern hybridization (Fig. 2, lanes 1 and 2). In contrast, laboratory strains lysogenized with phage 933W or H19J revealed single fragments which hybridized (Fig. 2, lanes 3 and 4). Independently of the *slt* genotype present in the individual strains, all O157 isolates possessed two or three restriction fragments hybridizing with the *p* gene probe (Fig. 2, lanes 5 to 12 and Table 3). Subsequent hybridization analyses were performed with *slt-I*, *slt-II*, and *slt-IIc* probes. *Sal*I fragments which hybridized with both the *p* gene probe and the distinct *slt* gene probes are shown in Table 3. Notably, all O157 strains possessed a common *Hin*dIII fragment of 30 kb and a common *Eco*RI fragment of 45 kb which hybridized with the *p* gene probe (Table 3). *Hin*dIII- and *Eco*RI-digested genomic DNA did not produce fragment bands hybridizing with both the *p* gene probe and the *slt-IB* or *slt-IIB* probe since the DNA regions between the *p* and *slt-IB* genes (not shown) and the *p* and *slt-IIB* genes possess an *Eco*RI restriction site (not shown) and a *Hin*dIII restriction site (see below).

PCR to generate fragments which contain *slt* and *p* gene sequences of *E. coli* O157 strains. To verify the linkage between *p* and *slt*, the PCR strategy described in Materials and Methods was applied. Oligonucleotide DK1, used in a PCR with either oligonucleotide KS8 or GK7, should result in amplification of fragments consisting of *p* and *slt-I* or *p* and *slt-II* sequences. As shown in Table 4, with every O157 strain possessing *slt-I* either alone or in combination with the *slt-II* or *slt-IIc* gene, a PCR product of 5.1 or 5.8 kb was obtained. Restriction analysis of the PCR products with *Pvu*II, shown in Fig. 3A, lanes 1 to 4, revealed two RFLPs represented by the profiles shown in lanes 1 and 2 to 4, respectively. Hybridization analysis with the *p* gene probe demonstrated that the PCR product contained *p* gene sequences (Fig. 3B, lanes 1 to 4).

By using the primer pair DK1-GK7 (Table 2), amplification products ranging from 6.3 to 7.2 kb were obtained when DNAs

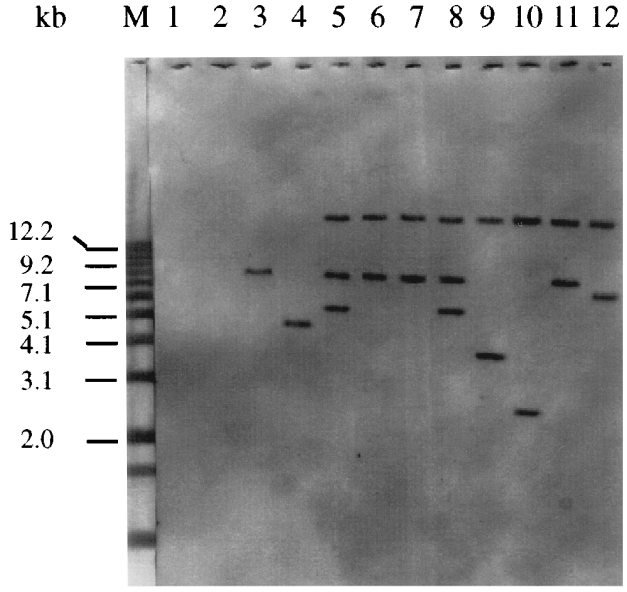

FIG. 2. Southern blot analysis of *Eco*RI-restricted total cellular DNAs from *E. coli* laboratory strains (lanes 1 to 4) and O157 clinical isolates (lanes 5 to 12) hybridized with the *p* gene probe. Genomic DNAs of the following strains were analyzed: DH5α (lane 1), C600 (lane 2), C600(H19J) (lane 3), C600(933W) (lane 4), 1249/87 (lane 5), 1658/91 (lane 6), 3978/91 (lane 7), 6537/91 (lane 8), 7513/91 (lane 9), 9167/91 (lane 10), 2785-2/93 (lane 11), and 4821/87 (lane 12). Lane M contained 1-kb DNA ladder (GIBCO BRL) as a molecular size standard.

TABLE 3. RFLP analyses with genomic DNA probed with *p* and distinct *slt* genes

Strain	*slt* genotype	Size(s) (kb) of *Sal*I fragment(s) hybridizing with probe:				Sizes (kb) of *Hin*dIII fragments hybridizing with *p* gene probe	Sizes (kb) of *Eco*RI fragments hybridizing with *p* gene probe
		slt-I	*slt-II*	*slt-IIc*	*p*		
6578/91	*slt-I*	27				15, 30	9, 45
1193/89	*slt-I*	27			27, 55	15, 30	9, 45
3574/92	*slt-II*		22		22, 55	17, 30	10, 45
5159/91	*slt-II*		27		22, 27, 35	7, 15, 30	4, 7, 45
7576/92	*slt-II*		22		22, 55	17, 30	10, 45
7513/91	*slt-IIc*			35	22, 35	15, 30	4, 45
2785-2/93	*slt-IIc*			55	22, 55	17, 30	9, 45
EDL933	*slt-I slt-II*	22	10		10, 22, 35	5, 15, 30	5.5, 9, 45
5291/92	*slt-I slt-IIc*	27		55	27, 55	15, 30	9, 45
9167/91	*slt-I slt-IIc*	27		55	27, 55	15, 30	2.7, 45
1249/87	*slt-II slt-IIc*		10	55	10, 22, 55	12, 17, 30	5.5, 9, 45
1658/91	*slt-II slt-IIc*		22	55	22, 55	17, 30	9, 45
3978/91	*slt-II slt-IIc*		22	55	22, 55	17, 30	9, 45
6537/91	*slt-II slt-IIc*		10	55	10, 22, 55	12, 17, 30	5.5, 9, 45

of strains containing *slt-II* either alone or together with *slt-I* or *slt-IIc* were used as targets (Table 4). Since *Pvu*II digestion yielded two bands not clearly separable, the PCR fragments were restricted with *Hin*dIII. Figure 3A, lanes 5 to 11, show the *Hin*dIII-digested PCR products of the strains listed in Table 4. The hybridization pattern obtained with the *p* gene probe is depicted in Fig. 3B, lanes 5 to 11.

The presence of the *slt-IB* or *slt-IIA* sequence in all of the PCR products shown in Table 4 was verified by sequencing a 300-bp sequence from the 3′ end.

PCR with non-O157 EHEC strains. Non-O157 EHEC serogroup O26 and O111 strains associated with HUS possessing either gene *slt-I* or *slt-II* were also subjected to PCR. The results depicted in Table 4 indicate that in the *slt-II*-harboring isolates, fragments similar in size to *slt-II*-harboring O157 strains were obtained. With strains possessing *slt-I*, the sizes of the fragments were distinct from those of *slt-I*-positive O157 strains.

Analysis of O157 strains which have undergone clonal turnover. Genomic DNAs of four O157 strains from four individual patients and their derivatives which had altered *slt* genotypes (Table 1) were hybridized with a probe which detects both *slt-IIB* and *slt-IIcB*. All initial strains yielded two hybridizing fragments (Fig. 4, lanes 1 to 4), whereas their genotypic derivatives showed only one fragment (lanes 5 to 8). To investigate whether phage sequences were simultaneously lost with the *slt-II* gene, we used the *p* gene probe in Southern blot analysis and observed that the original isolates contained three fragments hybridizing with *p*, whereas genomic DNAs of their derivatives had only two hybridizing fragments. A representative analysis of patterns obtained after digestion of the DNAs of strain 2785-1/93 and the derived genotypic variant 2785-2/93 with *Eco*RI, *Pvu*II, and *Kpn*I is shown in Fig. 5. PCR analysis demonstrated that strain 2785-2/93, which had lost *slt-II*, yielded negative results with the primer pair DK1-GK7 whereas 2785-1/93 was PCR positive (Table 4).

DISCUSSION

The observed nucleotide sequence similarity between the *p* genes of phages 933W and λ confirms that O157 phages are members of the lambdoid phage family (10, 22). For some individual O157 strains, it has been shown that the structural genes for SLT-I and SLT-II are located on temperate phages (9, 14, 20, 22, 26). Since all of the O157 strains investigated in this study revealed a close linkage between the *slt-I* or *slt-II* gene and phage gene *p*, it appears to be a general rule that genes encoding SLT-I or SLT-II are phage encoded in O157 strains. We also detected fragments which hybridized with both the *p* and the *slt-IIc* gene probes in *E. coli* O157 strains harboring *slt-IIc* alone but were unable to amplify a fragment. Oligonucleotide GK7 is complementary to the published *slt-IIcA* sequence (20), but the strains investigated in our study may possess distinct *p* sequences in the region where primer DK1 binds.

By Southern blot hybridization, we demonstrated that all of our O157 strains contain a highly conserved DNA fragment hybridizing with the gene *p* sequence but not with the *slt-I* or *slt-II* gene sequence. Whether this *p* gene is part of a defective phage or a phage which has any other function which increases the ability of O157 to survive or which influences the efficiency of its transmission is unknown.

The work of Rietra et al. (14) and Willshaw et al. (26) showed that the phages from serogroup O157 strains form a closely related group as indicated by their identical morphol-

TABLE 4. Results of PCR analysis with O157 and non-O157 EHEC strains

Strain	Serogroup	*slt* genotype	Size (kb) of PCR amplification product with primer pair:	
			DK1-KS8	DK1-GK7
6578/91	O157	*slt-I*	5.1	
1193/89	O157	*slt-I*	5.1	
3574/92	O157	*slt-II*		7.2
5159/91	O157	*slt-II*		7.2
7576/92	O157	*slt-II*		7.1
7513/91	O157	*slt-IIc*		
2785-2/93	O157	*slt-IIc*		
EDL933	O157	*slt-I slt-II*	5.8	6.3
5291/92	O157	*slt-I slt-IIc*	5.1	
1249/87	O157	*slt-II slt-IIc*		7.1
1658/91	O157	*slt-II slt-IIc*		7.2
2785-1/93	O157	*slt-II slt-IIc*		7.2
SF 493/93	O157	*slt-II*		7.1
SF 4162/94	O157	*slt-II*		7.1
E-D17	O26	*slt-I*	7.0	
E-D147	O26	*slt-II*		7.1
78/92	O111	*slt-I*	6.0	
1166/93	O111	*slt-II*		7.1

[a] Nucleotide sequences and primer sites are listed in Table 2.

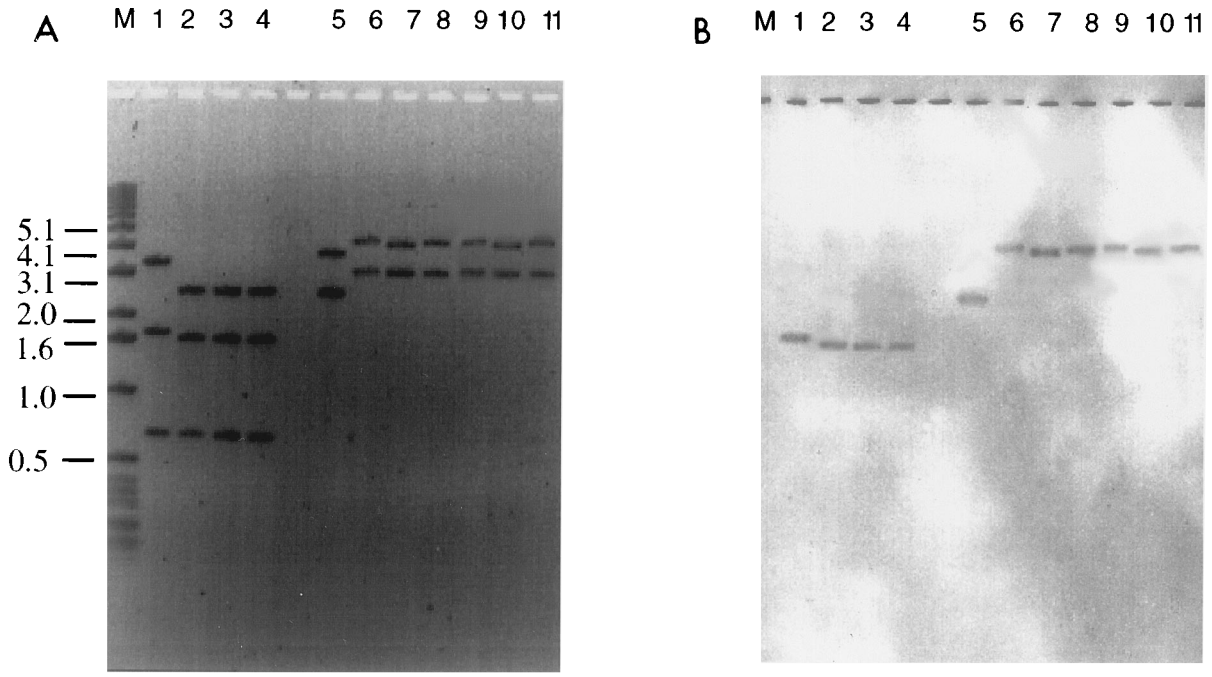

FIG. 3. Agarose gel electrophoresis (A) and Southern blot analysis with the *p* gene probe (B) of *Pvu*II-digested fragments from a PCR with primer pair DK1-KS8 (lanes 1 to 4) and *Hin*dIII-digested fragments resulting from a PCR with primer pair DK1-GK7 (lanes 5 to 11). PCR products from the following *E. coli* strains were analyzed: EDL933 (lanes 1 and 5), 6578/91 (lane 2), 1193/89 (lane 3), 5291/92 (lane 4), 3574/92 (lane 6), 7576/92 (lane 7), 5159/91 (lane 8), 1658/91 (lane 9), 1249/87 (lane 10), and 2785-1/93 (lane 11). Lane M contained a molecular size marker (1-kb DNA ladder; GIBCO BRL). The values to the left are molecular sizes in kilobase pairs.

ogies, genome sizes, and levels of DNA homology. Our findings revealed that the DNA region flanking *slt-I* was significantly different in size from the region flanking *slt-II*. Moreover, differences in the DNA fragments hybridizing with *p* and *slt* were observed, even within individual O157 strains. The various fragment sizes and restriction patterns of PCR products may possibly be useful for grouping of O157 strains as more strains become characterized.

Compared with plasmid typing, SLT genotyping, or phage typing, electrophoresis of genomic DNAs in which the DNA digests are subsequently hybridized with total λ DNA yields more highly discriminatory restriction patterns (12). The basis for this heterogeneity and the hybridization of many bands with λ DNA is not understood. Here, we show that O157 strains contain a minimum of two *p* genes. This explains the presence of multiple bands generated when hybridization of O157 DNA is performed with λ DNA. The PCR-generated *p* gene probe described here yields hybridization results which are easily interpretable and may be an alternative to probing with total λ DNA.

Since the possibility of treating O157 infections with antibiotics is limited, public health efforts directed to the prevention of O157 transmission are warranted (4). To clarify the mode of transmission, sensitive typing methods have been developed. Molecular methods have revealed that two criteria important for interpretation of typing results may be lacking in O157 typing (3, 8), namely, (i) that epidemiologically unrelated isolates should have different genotypes and (ii) that epidemiologically related isolates, e.g., from a single patient or outbreak, should have the same genotype. Analyses of genetic variation among O157 strains by multilocus enzyme electrophoresis have shown that O157 isolates belong to a single clone complex that is not closely related to SLT-producing strains of serotypes other than O157 (25). Therefore, EHEC O157 iso-

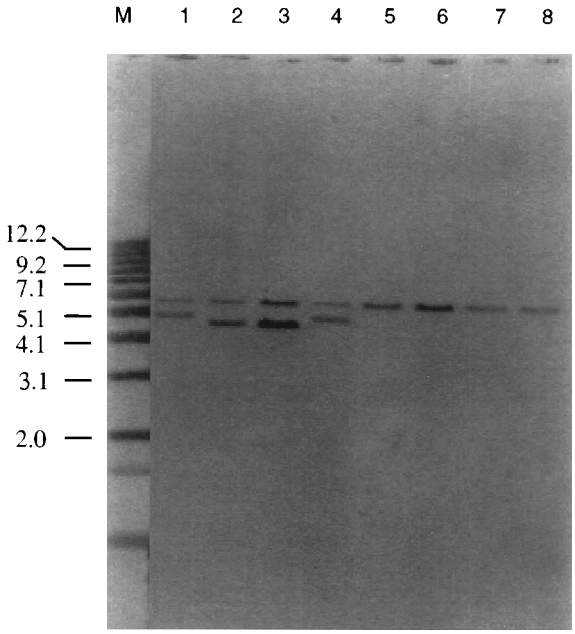

FIG. 4. Changes in clonal composition characterized by loss of the *slt-II* gene in O157 isolates from four patients. *Eco*RI-digested genomic DNAs were hybridized with a probe which detects both *slt-II* and *slt-IIc*. Genomic DNAs of the following strains were analyzed: 12-1/92 (lane 1), 47-1/93 (lane 2), 1567-1/93 (lane 3), 2785-1/93 (lane 4), 12-2/92 (lane 5), 47-2/93 (lane 6), 1567-2/93 (lane 7), and 2785-2/93 (lane 8). Lane M contained a molecular size standard (1-kb DNA ladder [GIBCO BRL]). The values to the left are molecular sizes in kilobase pairs.

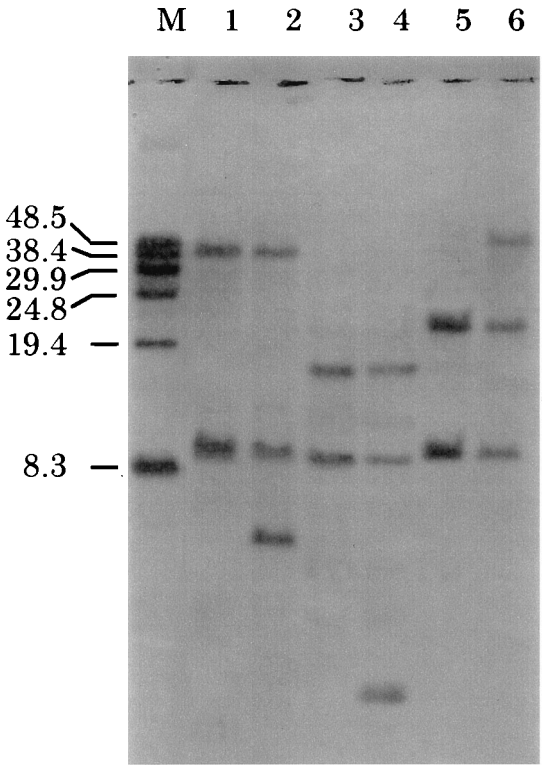

FIG. 5. Southern blot analysis of digested total cellular DNAs of *E. coli* 2785-1/93, harboring *slt-II* and *slt-IIc* (lanes 2, 4, and 6), and its derivative 2785-2/93, harboring only *slt-IIc* (lanes 1, 3, and 5), hybridized with the *p* gene probe. DNA was digested with the enzymes *Eco*RI (lanes 1 and 2), *Pvu*II (lanes 3 and 4), and *Kpn*I (lanes 5 and 6). Lane M contained the GIBCO BRL high-molecular-weight size marker. The values to the left are molecular sizes in kilobase pairs.

lates which are epidemiologically unrelated may have very similar or indistinguishable genotypes. In contrast to the above-described finding, *E. coli* O157 can, however, undergo rapid genotype alteration in the course of infection (8). This clonal turnover may also occur during food processing or when O157 changes hosts. As a result of clonal turnover, it may not be possible to demonstrate linkage among epidemiologically related O157 isolates. In this study, we demonstrated that altered RFLP patterns and *slt* genotypes result from simultaneous loss of both SLT-II-encoding genes and phage sequences. This loss is accompanied by rearrangement of bacterial DNA, which results in a different RFLP pattern. By restriction of genomic DNA with *Eco*RI, *Pvu*II, and *Kpn*I and subsequent hybridization with the *p* gene probe, this RFLP change is detectable and can assist in identifying strains which have undergone genotypic variation.

Although O157 has been intensively investigated during the last decade, there is still a lack of knowledge regarding the role of O157 phages in the epidemiology and pathogenesis of EHEC O157 infection. The genome of phage λ is 48.5 kb long, compared with approximately 70 kb for the O157 phages (26). It would be interesting to determine what type of information is encoded by the additional DNA present in the SLT-converting phage genomes. For example, are there other gene products important to O157 virulence? Further studies to characterize the O157 phages, such as determination of the insertion sites in the *E. coli* chromosome and investigation of whether the toxin genes can be transmitted from O157 to non-O157 by phages, are in progress. Lastly, by using the PCR techniques described here, SLT-converting phages may be rapidly detected in environmental samples such as contaminated water. It may be theoretically possible that ingestion of phages themselves, by either humans or animals, can cause infection of the existing *E. coli* flora.

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