JOURNAL OF CLINICAL MICROBIOLOGY, May 1993, p. 1200–1205
0095-1137/93/051200-06$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 31, No. 5

Clonal Structure and Pathogenicity of Shiga-Like Toxin-Producing, Sorbitol-Fermenting *Escherichia coli* O157:H$^-$

HELGE KARCH,[1]* HARTMUT BÖHM,[1] HERBERT SCHMIDT,[1] FLORIAN GUNZER,[1] STOJANKA ALEKSIC,[2] AND JÜRGEN HEESEMANN[1]

Institut für Hygiene und Mikrobiologie der Universität Würzburg, Bau 17, Josef-Schneider-Strasse 2, 8700 Würzburg,[1] and Nationales Zentrum für Enteritiserreger, Hygienisches Institut Hamburg, 2000 Hamburg,[2] Germany

Received 19 August 1992/Accepted 11 February 1993

We compared a collection of sorbitol-fermenting (SF) *Escherichia coli* O157:H$^-$ strains with SF *E. coli* O157:H45 and non-SF *E. coli* O157:H7 and *E. coli* O157:H$^-$ strains by pulsed-field gel electrophoresis. The SF *E. coli* O157:H$^-$ strains had identical or closely related *Xba*I patterns that differed markedly from those for the other *E. coli* O157 strains. Plasmid content and the presence of Shiga-like toxin-converting phages were determined for the SF *E. coli* O157:H$^-$ strains, indicating that these strains harbor a single 90-kb plasmid. They are lysogenized by toxin-converting phages and harbor the *eae* gene. Nonmotile *E. coli* O157 strains were observed to adhere more efficiently to HEp-2 cells than the motile strains. From their phenotypic and genotypic features, the SF *E. coli* O157:H$^-$ strains may well represent a new clone with non-SF *E. coli* O157:H7 pathogenic characteristics.

By using DNA probes complementary to Shiga-like toxin (SLT) (verocytotoxin) genes and subsequently serotyping probe-positive colonies, we have recently identified sorbitol-fermenting (SF) β-glucuronidase-positive *Escherichia coli* O157:H$^-$ strains which were previously shown to produce SLT-II (1, 13). SF *E. coli* O157:H$^-$ strains were found to represent the most frequent SLT-producing serotype recovered from stool specimens of patients with hemolytic-uremic syndrome in Germany (8). These findings are at variance with the predominance of non-SF (NSF) *E. coli* O157:H7 isolates reported in North America and the British Isles (14, 19). NSF *E. coli* O157:H7 strains do not ferment sorbitol within 48 h of incubation, and they show a negative reaction for β-glucuronidase (7, 16). Previous analyses of epidemiologically unrelated NSF *E. coli* O157:H7 strains have indicated that these isolates are lysogenized by toxin-converting phages (17, 20) and harbor a high-molecular-weight plasmid (12, 22). Moreover, they possess a chromosomal gene, the *eae* gene, which appears to be involved in attaching and effacing lesions (25). Multilocus enzyme electrophoresis and DNA fingerprinting revealed that NSF *E. coli* O157:H7 strains from different geographical areas are of the same lineage (3, 23, 24). In this study, we analyzed SF *E. coli* O157:H$^-$ strains by pulsed-field gel electrophoresis (PFGE) to estimate their genetic relatedness to one another and to NSF *E. coli* O157:H7 strains. In addition, the plasmid profile of the SF *E. coli* O157:H$^-$ strains was determined and the presence of SLT-converting phages was assessed. Furthermore, their characteristics of adherence to HEp-2 cells were examined. Evidence that the SF *E. coli* O157:H$^-$ strains represent a new clone within *E. coli* serogroup O157 is presented.

(Part of this work will appear in the M.D. theses of H. Böhm and F. Gunzer, University Würzburg, Würzburg, Germany.)

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. A total of 21 SF *E. coli* O157:H$^-$ strains were examined. Seventeen were from a previous report (8). All isolates stem from different individuals. Twelve SF *E. coli* O157:H$^-$ strains were from children with hemolytic-uremic syndrome treated in 12 different pediatric centers throughout Germany; they were isolated between 1988 and 1991. As far as we know, there was no contact between any of these 12 patients. The remaining nine SF *E. coli* O157:H$^-$ strains were isolated from patients in two other clinics. Twelve NSF *E. coli* O157:H7 strains were from patients with hemolytic-uremic syndrome who were also selected from different geographical locations in Germany, so that interpatient contact could likewise be precluded in this separate group. One SLT-I-producing NSF *E. coli* O157:H7 isolate (strain 1034/89) from a patient with enterocolitis was available for comparison. Twelve NSF *E. coli* O157:H$^-$ strains from patients suffering from enterocolitis, hemorrhagic colitis, and hemolytic-uremic syndrome were also included. Ten of these strains were described previously (1). Moreover, we tested four nontoxinogenic SF strains of *E. coli* O157:H45 from patients with enterocolitis. These strains were identified by slide agglutination with O157 antiserum. The control strains employed in the adhesion assays and polymerase chain reaction (PCR) studies were the *E. coli* O127:H6 strain E2348/69, the NSF *E. coli* O157:H7 strain EDL 933, the NSF *E. coli* O157:H$^-$ strain E32511, and the laboratory strain *E. coli* C600. These strains were grown in Trypticase soy broth for analysis in PFGE and PCR or in brain heart infusion broth when used in the adherence assays. *E. coli* DH5α was used in the cloning studies.

Plasmid analysis. Plasmid DNA was isolated as described previously (12) and analyzed by vertical agarose gel electrophoresis with 0.8% agarose gels. The plasmid preparations were compared by restriction enzyme analysis with *Hin*dIII, *Eco*RI, and *Bam*HI (Boehringer, Mannheim, Germany).

Isolation of toxin-converting phages and detection of *slt* genes. Cultures were induced by UV light, and phages present in the UV light-induced lysates were detected by the formation of plaques on the *E. coli* K-12 indicator strain

W3110. Phages were tested for their capacity to convert the indicator strain to an SLT-producing strain. These experiments and the cytotoxicity assay were performed as described previously (11). The presence of *slt* genes was demonstrated by PCR analysis with primers MK1-MK2 as described previously (3).

PFGE. Genomic DNA used for PFGE was isolated by the procedure described previously (3) and was cleaved with restriction enzymes *Xba*I and *Not*I (New England Biolabs, Schwalbach, Germany) according to the manufacturer's instructions. In order to determine the relationships among the different strains, we first compared numbers and sizes of fragments, with all strains being compared with one another. PFGE analysis was conducted three times with all of the strains. Hybridization of PFGE-separated DNA was carried out with a 230-bp fragment of the *slt-II* gene (3). This fragment was used as a hybridization probe following labelling with digoxigenin. Probe labelling, hybridization, and detection were performed as described previously (3).

PCR for amplification of *eae* gene fragments. A PCR designed to amplify a conserved region of the *eae* gene seen at the 5′ ends of the enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* structural genes (9, 25) was conducted with primers targeted to the *eae* gene of *E. coli* E2348/69 (9). In a 50-μl PCR assay containing 50 pmol of each of the primers (5′-CCC GAA TTC GGC ACA AGC ATA AGC-3′ and 5′-CCG GAT CCG TCT CGC CAG TAT TCG-3′), an 864-bp fragment was amplified. The suspensions containing about 10^3 bacteria were incubated at 94°C for 30 s to denature the DNA, at 52°C for 1 min to anneal primers, and at 72°C for 2 min to extend annealed primers. Amplification was carried out for 30 cycles. The identity of the 864-bp fragments was confirmed by sequence analysis of a 200-bp subfragment. Following purification of the PCR fragments from strains E2348/69 and EDL 933 and the SF *E. coli* O157:H⁻ strain 703/88, these fragments were ligated into vector pUC18 and the plasmids were used to transform *E. coli* DH5α. From each of the genes, both strands and two independent PCR-derived clones were sequenced. For routine screening of *eae* genes in the *E. coli* O157 strains, the identity of the 864-bp fragments was corroborated by restriction analysis with *Pst*I, which cuts the 864-bp fragment twice, thereby generating three fragments (372, 249, and 243 bp) in accordance with the published DNA sequence (9).

Adhesion assays and FAS test. The capabilities of strains belonging to the various O157 groups and control strains to adhere to HEp-2 cells were tested as described by Cravioto et al. (6) with minor modifications. Briefly, bacteria used for the adhesion assay were grown in brain heart infusion broth overnight. The HEp-2 cells were grown for 24 h on glass coverslips located in minimum essential medium containing tissue culture plates. The cells were washed three times in minimum essential medium prior to being incubated in 1 ml of medium containing 2% fetal calf serum. A sample (10 μl) of the bacteria was incubated for 3 and 6 h at 37°C with HEp-2 cells. Subsequently, the monolayer was washed (two times) with phosphate-buffered saline (PBS) and the cells plus adherent bacteria were fixed for 10 min with 200 μl of 3.7% formaldehyde in PBS (pH 7.4). Following this, the coverslips were washed in PBS (three times) and incubated for 5 min with 500 μl of 0.1% Triton X-100 in PBS.

The fluorescent actin-staining (FAS) test was realized by a modification of the procedure described by Knutton et al. (15). For fluorescence staining, we used *N*-7-nitrobenz-2-oxa-1,3-diazole–phallacidin (NBD-phallacidin; Molecular Probes, Nordwald, Germany) (5). Briefly, after three washes in PBS the coverslips were treated with NBD-phallacidin dissolved in a 1.5-ml methanol solution to contain 1 U in 5 μl of methanol. This amount was used for staining one coverslip. After the methanol was evaporated, 200 μl of PBS was added and incubated with the slips for 20 min at room temperature in a darkened chamber. The slips were washed twice in PBS, air dried, and mounted in glycerol PBS. They were examined by light fluorescence with a Leitz microscope. Each FAS test comprised examination of 100 HEp-2 cells selected at random and determination of the percentage of cells with actin fluorescence.

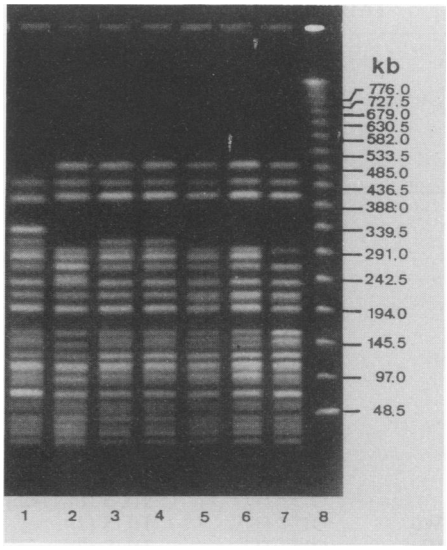

FIG. 1. Agarose gel showing *Xba*I digestion patterns of SF *E. coli* O157:H⁻ strains isolated in 1988 (lanes 1 and 2), 1989 (lanes 3 and 4), 1990 (lanes 5 and 6), and 1991 (lane 7) from patients with hemolytic-uremic syndrome who were all at different geographical locations in Germany. Lambda DNA concatemers were used as DNA size markers (lane 8). Sizes (in kilobases) are at the right.

RESULTS

PFGE of *E. coli* O157 isolates. The genotypic relatedness of 21 SF *E. coli* O157:H⁻ strains from individual patients was investigated by restriction endonuclease analysis of chromosomal DNA separated by PFGE. The *Xba*I digestion patterns of seven SF *E. coli* O157:H⁻ strains are shown in Fig. 1, lanes 1 to 7. With regard to fragment sizes and numbers of fragment bands, the isolates obtained between 1988 and 1991 were very similar. To estimate the genetic relatedness in quantitative terms, we compared the number and size of the fragment bands in each of the 21 SF *E. coli* O157:H⁻ strains. All strains were compared with each other, and the numbers of fragments not identical to those in the SF *E. coli* O157:H⁻ strain 703/88 are shown for representative strains in Table 1. The *Xba*I patterns of the O157:H⁻ isolates from the individual cases were highly related, differing at a maximum in five fragment bands (Table 1 and Fig. 1, lanes 1 to 7). Altogether, the numbers and sizes of the fragment bands in three strains were identical; four strains differed in one fragment, eight strains differed in two fragments, four strains differed in three fragments, and one strain differed in five fragments in a comparison of the number of fragment sizes different from those in the SF *E. coli* O157:H⁻ strain 703/88 (data not shown). By contrast, the *Xba*I patterns of these strains

TABLE 1. Phenotypic and genotypic characteristics of representative *E. coli* O157 isolates used in this study[a]

Strain[b]	Serotype	Disease strain is associated with (source or reference)	Results for:		Result from indicated comparison with strain 703/88	
			eae/slt[c]	Sorbitol fermentation/ β-glucuronidase activity[d]	Numeric difference in fragments	No. of nonidentical fragments
703/88	O157:H$^-$	HUS (1)	+/+	+/+	0	0
425/88	O157:H$^-$	HUS (1)	+/+	+/+	+2	5
493/89	O157:H$^-$	HUS (this study)	+/+	+/+	+2	2
210/89	O157:H$^-$	HUS (1)	+/+	+/+	−1	3
1042/90	O157:H$^-$	HUS (this study)	+/+	+/+	+2	2
817/90	O157:H$^-$	HUS (1)	+/+	+/+	+1	2
658/91	O157:H$^-$	HUS (this study)	+/+	+/+	+1	3
514/91	O157:H$^-$	HUS (this study)	+/+	+/+	+1	1
243/88	O157:H$^-$	EC (this study)	+/+	−/−	−1	17
427/89	O157:H$^-$	HC (1)	+/+	−/−	−2	18
1087/90	O157:H$^-$	HC (1)	+/+	−/−	−2	20
257/91	O157:H$^-$	EC (1)	+/+	−/−	−2	17
669/88	O157:H7	HUS (this study)	+/+	−/−	−2	14
3978/89 Wü	O157:H7	HUS (2)	+/+	−/−	−4	18
3268/90 Wü	O157:H7	HUS (2)	+/+	−/−	−4	18
715/91 Wü	O157:H7	HUS (this study)	+/+	−/−	−4	18
202/88	O157:H45	EC (this study)	+/−	+/+	−2	16
966/89	O157:H45	EC (this study)	+/−	+/+	−2	18
904/90	O157:H45	EC (1)	+/−	+/+	−3	16
006/91	O157:H45	EC (this study)	+/−	+/+	−2	14

[a] H$^-$, nonmotile; HUS, hemolytic-uremic syndrome; EC, enterocolitis; HC, hemorrhagic colitis.
[b] Numbers after the shill in strain names are the years of isolation.
[c] Performed by PCR analysis with primers complementary to *slt* or *eae* gene sequences. +, positive; −, negative.
[d] +, positive after 24 h; −, negative after 24 h.

differed from those of NSF *E. coli* O157:H7 and *E. coli* O157:H$^-$ strains and SF *E. coli* O157:H45 strains markedly (Table 1). The difference in the PFGE patterns of SF *E. coli* O157:H$^-$ and NSF *E. coli* O157:H7 is also illustrated in Fig. 2A, in which two exemplary strains of NSF *E. coli* O157:H7 (Fig. 2A, lanes 1 and 2) are compared with three strains of SF *E. coli* O157:H$^-$ (Fig. 2A, lanes 3 to 5).

*Xba*I fragments separated by PFGE and hybridized with a probe derived from the *slt-II* gene revealed specific binding to a 210-kb fragment in all three SF *E. coli* O157:H$^-$ strains (Fig. 2B, lanes 3 to 5) and binding of the *slt-II* probe to a fragment of 530 kb in the NSF *E. coli* O157:H7 strain (Fig. 2B, lane 1). The SLT-I-producing *E. coli* O157:H7 strain 1034/89 did not hybridize (Fig. 2B, lane 2).

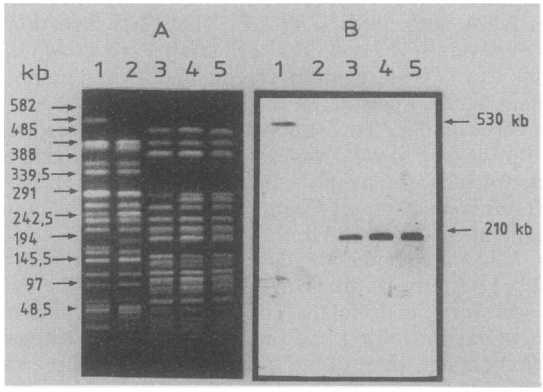

FIG. 2. *Xba*I-cleaved genomic DNA from an SLT-II-producing *E. coli* O157:H7 strain (lanes 1), an SLT-I-producing *E. coli* O157:H7 strain (lanes 2), and SLT-II-producing SF *E. coli* O157:H$^-$ strains (lanes 3 to 5) separated by PFGE (A) and Southern hybridization with a DNA probe specific for *slt-II* (B). Molecular sizes (in kilobases) are indicated.

Phage induction. Twelve randomly selected SF *E. coli* O157:H$^-$ strains were tested for the presence of toxin-converting phages after treatment with UV light. Plaques were observed in all cases. The toxic activity in the laboratory strain W3110 was confirmed after transduction with phages from two of the SF *E. coli* O157:H$^-$ strains.

Plasmid profile. All 21 SF *E. coli* O157:H$^-$ strains contained only a single plasmid of 90 kb on agarose gels. A plasmid of a similar size was present in all 12 NSF *E. coli* O157:H7 isolates investigated and in all 12 NSF *E. coli* O157:H$^-$ strains. In addition, 6 of the 12 NSF *E. coli* O157:H7 isolates and 8 of 12 NSF *E. coli* O157:H$^-$ strains possessed a 6.6-kb plasmid. Restriction enzyme analysis of the 90-kb plasmids revealed that those from SF *E. coli* O157:H$^-$, NSF *E. coli* O157:H$^-$, and NSF *E. coli* O157:H7 strains share fragments of similar sizes.

PCR analysis for the *eae* gene. Since the *eae* gene has been previously shown to be present in *E. coli* O157:H7 isolates (2, 25), we now analyzed the strains listed in Table 1 for this gene using PCR. An 864-bp fragment was present in all strains, including the SLT-negative *E. coli* O157:H45 isolates (Table 1). No fragments were present in *E. coli* C600 by PCR analysis. The identity of the 864-bp fragment was shown by restriction enzyme digestion with *Pst*I, yielding a 372-bp fragment and two smaller fragments not separable on this gel system (Fig. 3, lane 2). Identical fragment sizes were seen when the *eae* gene-positive reference strains EDL 933 and E2348/69 were amplified and digested with *Pst*I (Fig. 3, lanes 4 and 6). The PCR products from all three strains were cloned and a 200-bp fragment was sequenced to further

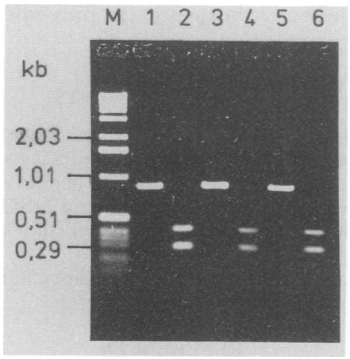

FIG. 3. Demonstrations of *eae* gene sequences in the SF *E. coli* O157:H⁻ strain 703/88 and in the control strains EDL 933 and E2348/69 by PCR analysis with primers targeted to a conserved region of the *eae* gene. In lanes 1, 3, and 5, the fragments obtained after PCR with strains 703/88 (lane 1), EDL 933 (lane 3), and E2348/69 (lane 5) are shown. Digestion of the PCR products from the respective strains with *Pst*I was performed, and the DNA was separated in this gel (lanes 2, 4, and 6). *Pst*I cuts the 864-bp fragment into a 372-bp fragment and two smaller fragments (243 and 249 bp) which could not be separated in this gel system. Lane M, molecular weight markers (1-kb DNA ladder) (GIBCO BRL).

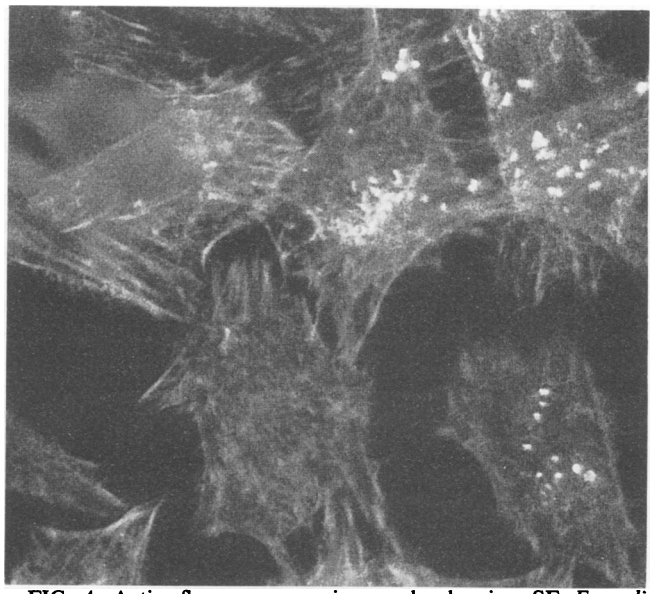

FIG. 4. Actin fluorescence micrograph showing SF *E. coli* O157:H⁻ strain 703/88-infected HEp-2 cells. Actin fluorescence is localized mainly at the cell periphery at positions with adherent bacteria.

prove its identity. Only 2 nucleotide bases were distinct in the subfragment when the sequence from the SF *E. coli* O157:H⁻ strain 703/88 was compared with that previously published for E2348/69 (9).

Adhesion assay and FAS test. Subsequent to *eae* gene identification, we tested the capacities of three representative SF *E. coli* O157:H⁻ strains, three NSF *E. coli* O157:H⁻ strains, three NSF *E. coli* O157:H7 strains, three *E. coli* O157:H45 strains, and two strains (E2348/69 and E32511) presently being studied to adhere to HEp-2 cells (15) and cause a positive FAS test. A negative control strain (*E. coli* C600) was also included. The nonmotile SF and NSF *E. coli* O157:H⁻ strains were positive with HEp-2 cells in both 3- and 6-h FAS tests. Following 3-h incubation times, the percentage of HEp-2 cells with fluorescence was at least 50%. After a 6-h incubation period, more than 80% of the HEp-2 cells exhibited fluorescence. An example is shown in Fig. 4. This figure shows that nearly all of the cells contain intense spots of fluorescence which correspond in size and position with adherent bacteria. In contrast, under the same conditions with the SF *E. coli* O157:H7 and SF *E. coli* O157:H45 strains fluorescence was seen only in the 6-h test. Phase-contrast micrographs showed that about 5 to 10% of the HEp-2 cells harbored bacteria in low numbers (5 to 10 bacteria). The NSF *E. coli* O157:H⁻ strain E32511 used here because Knutton et al. reported good adherence (15) was shown to adhere as well as the SF *E. coli* O157:H⁻ and nonmotile NSF *E. coli* O157:H⁻ strains from our collection.

DISCUSSION

SLT-producing SF *E. coli* O157:H⁻ strains were first discovered in an outbreak of hemolytic-uremic syndrome in children that occurred in Upper Bavaria, Germany, in 1988 (13). Because these strains ferment sorbitol within 24 h, they are not detectable on Sorbitol MacConkey agar, which is usually recommended for identifying NSF *E. coli* O157:H7. Their true significance was demonstrated by Gunzer et al., who have shown that they represent the most prevalent pathogen in children with hemolytic-uremic syndrome in Germany (8). For this reason, we wanted to clarify their virulence factors and determine whether the SF *E. coli* O157:H⁻ strains are phenotypical variants of the NSF *E. coli* O157:H7 clone or whether they are variants of a clone different from this. We discovered that SF *E. coli* O157:H⁻ and NSF *E. coli* O157:H7 strains have a 90-kb plasmid in common, along with toxin-converting phages and the *eae* gene. Adherence to HEp-2 cells is well developed in nonmotile *E. coli* O157 strains.

The results from the FAS test indicated that the motile *E. coli* O157 isolates (both *E. coli* O157:H7 and *E. coli* O157:H45 isolates) adhere only in the 6-h test, and they adhere to few cells bearing small numbers of bacteria. In direct contrast to this, the nonmotile *E. coli* O157 isolates (both SF and NSF) show attachments of large numbers of bacteria in the 3- and 6-h tests. It may be assumed from these data that flagella interfere with the adherence of motile strains to the HEp-2 cells. Because the flagellar and nonflagellar strains probably differ in characteristics other than just the presence of flagella, additional experimentation with isogenic strains differing only in the presence of flagella will be necessary.

In the literature, the term "EHEC" has been mostly associated with NSF *E. coli* O157:H7, presumably because these strains have been isolated almost exclusively from outbreaks of hemorrhagic colitis and hemolytic-uremic syndrome (14). Our results indicate that NSF *E. coli* O157:H7 strains are not unique in their virulence potential, as the SF *E. coli* O157:H⁻ strains are equally capable of causing an outbreak of hemolytic-uremic syndrome (12) as well as other hitherto-unpublished outbreaks (10). The 90-kb plasmid which seems to support colonization (21) and the *eae* gene which is said to be involved in attaching and effacing lesions (9, 25) might well contribute to the virulence potential of SF *E. coli* O157:H⁻. The 6.6-kb plasmid previously shown to encode a colicin D-like colicin termed Col_{D157} (4) in NSF *E. coli* O157:H7 isolates was surprisingly not present in any SF *E. coli* O157:H⁻ strains. It became apparent that SF *E. coli* O157:H⁻ strains were shown to be lysogenized by phages

that harbor *slt-II* genes. Other investigators have also made the observation that SLT-II has been more strongly associated with systemic sequelae than has SLT-I (18). In accordance with the findings of Barrett et. al (2), we have discovered the *eae* gene in all NSF *E. coli* O157:H7 strains; however, we have further shown its presence in SF *E. coli* O157:H$^-$ and *E. coli* O157:H45 strains. Two groups of *eae* genes from enteropathogenic *E. coli* and EHEC have been sequenced to date (9, 25), and it remains to be seen whether the *eae* gene found in nontoxinogenic *E. coli* O157:H45 is related to the EHEC or enteropathogenic *E. coli eae* gene.

PFGE was found to be a highly reliable technique, with repetitive analysis of the SF *E. coli* O157:H$^-$ strains resulting in reproducible fingerprints for each strain analyzed. Macrorestriction analysis proved useful for identifying individual strains. Eighteen of 21 SF *E. coli* O157:H$^-$ strains could be identified by their own individual fingerprints. In addition, PFGE analysis clearly showed that the SF *E. coli* O157:H$^-$ strains are more closely related to one another than to the NSF *E. coli* O157:H$^-$ and the *E. coli* O157:H7 strains. Here we ask whether the SF *E. coli* O157:H$^-$ strains represent a new clone and if so, how much PFGE variability should be acceptable for a clone. Of 34 NSF *E. coli* O157:H7 strains recently examined by PFGE, a total of 26 had individual *Xba*I patterns (2). After patterns of restriction fragment length were evaluated in quantitative terms by determining the number of fragments and the size of each fragment in each individual strain, the deviation between the bands of the various strains was slight. In the majority of NSF *E. coli* O157:H7 strains, one to three fragment bands differed, with the maximal difference in one strain being eight fragments. By contrast, a total of 17 bands were different in a comparison of nontoxinogenic SF *E. coli* O157:H19 and H43 strains (2). These results showed that NSF *E. coli* O157:H7 strains have identical or closely related PFGE patterns and are therefore believed to belong to one clonal descent, a finding also supported by multilocus enzyme electrophoresis (23, 24). In this context, we mention that macrorestriction analysis estimates the genetic relatedness of strains from the distribution of infrequent oligonucleotide sequences in the chromosome. The variety of the PFGE patterns depends on the restriction endonuclease applied. By comparing four rare cutting restriction enzymes (*Not*I, *Sfi*I, *Pac*I, and *Xba*I) to conduct PFGE analysis of NSF *E. coli* O157:H7 strains, we found that *Xba*I yielded the greatest polymorphism among strains with a clear-cut resolution of bands (3). From the PFGE data presented here, we postulate that SF *E. coli* O157:H$^-$ strains, by being very similar to each other in the PFGE analysis, are clonal in nature but that, by being particularly different in PFGE patterns from NSF *E. coli* O157:H7, they represent a completely different clone.

What does clonality of SF *E. coli* O157:H$^-$ mean in practical terms? We present three possibilities to explain this phenomenon: (i) SF *E. coli* O157:H$^-$ has its origin from the same source, (ii) there might have been cross-infections between the patients (which we were not aware of), or (iii) recent and rapid propagation prevented sequence variations. Unfortunately, these questions cannot be answered at present, because the epidemiology of these pathogens has not been shown at all. By using molecular probes, we have analyzed more than 10,000 single colonies from 580 bovine beef, feces, and milk samples described as potential reservoirs for NSF *E. coli* O157:H7 (13) and have not been able to demonstrate SF *E. coli* O157:H$^-$. Further studies are essential in order to identify the reservoirs of these pathogens and to implement effective measures to prevent infections and hinder global propagation.

ACKNOWLEDGMENTS

We thank Sylvia Scotland and Bernhard Rowe (Central Public Health Laboratory Service, London, United Kingdom) for confirming two strains of the new clone described here as serogroup O157, nonmotile, sorbitol fermenting, verocytotoxin 2 (SLT-II), and β-glucuronidase positive. We thank Beatrix Henkel for excellent technical assistance.

This work was supported by Deutsche Forschungsgemeinschaft grant Ka 717-2.

REFERENCES

1. **Aleksic, S., H. Karch, and J. Bockemühl.** 1992. A biotyping scheme for Shiga-like (Vero) toxin-producing *Escherichia coli* O157 and a list of serological cross-reactions between O157 and other gram-negative bacteria. Zentralbl. Bakteriol. **276:**221–230.
2. **Barrett, T. J., J. B. Kaper, A. E. Jerse, and I. K. Wachsmuth.** 1992. Virulence factors in Shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. J. Infect. Dis. **165:**979–980.
3. **Böhm, H., and H. Karch.** 1992. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J. Clin. Microbiol. **30:**2169–2172.
4. **Bradly, D. E., and S. P. Howard.** 1991. Colicinogeny of O157:H7 enterohemorrhagic *Escherichia coli* and the shielding of colicin and phage receptors by their O-antigenic side chains. Can. J. Microbiol. **37:**97–104.
5. **Clerc, P., and P. J. Sansonetti.** 1987. Entry of *Shigella flexneri* into HeLa cells: evidence for directed phagocytosis involving actin polymerization and myosin accumulation. Infect. Immun. **55:**2681–2688.
6. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. **3:**95–99.
7. **Doyle, M. P., and J. L. Schoeni.** 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. **48:**855–856.
8. **Gunzer, F., H. Böhm, H. Rüssmann, M. Bitzan, S. Aleksic, and H. Karch.** 1992. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J. Clin. Microbiol. **30:**1807–1810.
9. **Jerse, A. E., J. Yu, B. D. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Natl. Acad. Sci. USA **87:**7839–7843.
10. **Karch, H.** Unpublished data.
11. **Karch, H., J. Heesemann, and R. Laufs.** 1987. Phage-associated cytotoxin production by and enteroadhesiveness of enteropathogenic *Escherichia coli* isolated from infants with diarrhea in West Germany. J. Infect. Dis. **155:**707–715.
12. **Karch, H., J. Heesemann, R. Laufs, A. D. O'Brien, C. O. Tacket, and M. M. Levine.** 1987. A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect. Immun. **55:**455–461.
13. **Karch, H., R. Wiss, H. Gloning, P. Emmrich, S. Aleksic, and J. Bockemühl.** 1990. Hämolytisch-urämisches Syndrom bei Kleinkindern durch Verotoxin-produzierende *Escherichia coli*. Dtsch. Med. Wochenschr. **115:**485–495.
14. **Karmali, M.** 1989. Infection by verocytotoxin-producing *Escherichia coli*. Clin. Microbiol. Rev. **2:**15–38.
15. **Knutton, S., T. Baldwin, P. H. Williams, and S. McNeish.** 1989. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect. Immun. **57:**1290–1298.
16. **March, S. B., and S. Ratnam.** 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869–872.

17. **O'Brien, A. D., J. W. Newland, S. F. Miller, R. K. Holmes, H. W. Smith, and S. B. Formal.** 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science **226:**694–696.
18. **Ostroff, S. M., P. I. Tarr, M. A. Neill, J. H. Lewis, N. Hargrett-Bean, and J. M. Kobayashi.** 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J. Infect. Dis. **160:**994–998.
19. **Scotland, S. M., B. Rowe, H. R. Smith, G. A. Willshaw, and R. J. Gross.** 1988. Vero cytotoxin-producing strains of *Escherichia coli* from children with hemolytic uremic syndrome and their detection by specific DNA probes. J. Med. Microbiol. **25:**237–243.
20. **Smith, H. R., N. D. Day, M. Scotland, R. J. Gross, and B. Rowe.** 1984. Phage-determined production of vero cytotoxin in strains of *Escherichia coli* serogroup O157. Lancet **ii:**1242–1243.
21. **Wadlowski, E. A., L. M. Sung, J. A. Burris, J. E. Samuel, and A. D. O'Brien.** 1990. Acute renal tubular necrosis and death of mice orally infected with *Escherichia coli* strains that produce Shiga-like toxin type II. Infect. Immun. **58:**3959–3965.
22. **Wells, J. G., B. R. Davis, I. K. Wachsmuth, L. W. Riley, R. S. Remis, R. Sokolow, and G. K. Morris.** 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. **18:**512–520.
23. **Whittam, T. S., I. K. Wachsmuth, and R. A. Wilson.** 1988. Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic uremic syndrome. J. Infect. Dis. **157:**1124–1133.
24. **Whittam, T. S., and R. A. Wilson.** 1988. Genetic relationships among pathogenic *Escherichia coli* of serogroup O157. Infect. Immun. **56:**2467–2473.
25. **Yu, J., and J. B. Kaper.** 1992. Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. **6:**411–417.