Applied and Environmental Microbiology, Jan. 2008, p. 67–72 Vol. 74, No. 1
0099-2240/08/$08.00+0 doi:10.1128/AEM.01906-07

Recycling of Shiga Toxin 2 Genes in Sorbitol-Fermenting Enterohemorrhagic *Escherichia coli* O157:NM[▽]

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Received 19 August 2007/Accepted 25 October 2007

Using colony blot hybridization with *stx*$_2$ and *eae* probes and agglutination in anti-O157 lipopolysaccharide serum, we isolated *stx*$_2$-positive and *eae*-positive sorbitol-fermenting (SF) enterohemorrhagic *Escherichia coli* (EHEC) O157:NM (nonmotile) strains from initial stool specimens and *stx*-negative and *eae*-positive SF *E. coli* O157:NM strains from follow-up specimens (collected 3 to 8 days later) from three children. The *stx*-negative isolates from each patient shared with the corresponding *stx*$_2$-positive isolates *fliC*$_{H7}$, non-*stx* virulence traits, and multilocus sequence types, which indicates that they arose from the *stx*$_2$-positive strains by loss of *stx*$_2$ during infection. Analysis of the integrity of the *yecE* gene, a possible *stx* phage integration site in EHEC O157, in the consecutive *stx*$_2$-positive and *stx*-negative isolates demonstrated that *yecE* was occupied in *stx*$_2$-positive but intact in *stx*-negative strains. It was possible to infect and lysogenize the *stx*-negative *E. coli* O157 strains in vitro using an *stx*$_2$-harboring bacteriophage from one of the SF EHEC O157:NM isolates. The acquisition of the *stx*$_2$-containing phage resulted in the occupation of *yecE* and production of biologically active Shiga toxin 2. We conclude that the *yecE* gene in SF *E. coli* O157:NM is a hot spot for excision and integration of Shiga toxin 2-encoding bacteriophages. SF EHEC O157:NM strains and their *stx*-negative derivatives thus represent a highly dynamic system that can convert in both directions by the loss and gain of *stx*$_2$-harboring phages. The ability to recycle *stx*$_2$, a critical virulence trait, makes SF *E. coli* O157:NM strains ephemeral EHEC that can exist as *stx*-negative variants during certain phases of their life cycle.

Sorbitol-fermenting (SF) enterohemorrhagic *Escherichia coli* (EHEC) O157:NM strains (nonmotile) were first identified in 1988 in Germany (28) and have now emerged as a cause of human diseases, including life-threatening hemolytic-uremic syndrome (HUS), in additional countries (4, 7, 8, 17, 20, 22, 37). In Germany, SF EHEC O157:NM strains account for >10% of sporadic cases of HUS (20, 22), and after EHEC O157:H7, they are the second most common cause of HUS (20, 22). They have also caused several outbreaks in Germany (5, 41, 42) and the United Kingdom (3, 18).

The genes encoding Shiga toxins (Stx) (44), the major virulence factors of EHEC, are located in temperate lambdoid bacteriophages which are integrated in the host genome during lysogenic growth (2, 25, 29, 35, 48, 53). A lytic phage cycle can be induced in EHEC strains using sublethal doses of UV light (35) or subtherapeutic concentrations of various antibiotics (23, 33, 35, 45, 47, 58). The existence of *stx* genes in a multitude of *E. coli* serotypes (2, 55) is attributable to transduction with *stx*-converting phages (2, 25, 45). Transduction in vivo has been demonstrated in several animal models including mice (1), sheep (14), and insects (39).

Both loss and transfer of the *stx* gene appear to occur during human infection and can lead to a change in the pathotype of the infecting strain (10, 21, 34). We have demonstrated the excretion of *stx*$_2$-positive EHEC O157:NM in the initial stool specimen (collected 8 days after the onset of prodromal diarrhea) of an HUS patient, followed by the excretion of *stx*-negative *E. coli* O157:NM in the follow-up stool specimen collected 3 days later (34). Comparison of *stx* gene losses in SF EHEC O157:NM and non-SF EHEC O157:H7 isolates showed a significantly higher proportion of *stx*-negative strains (12.7% versus 0.8%) among SF *E. coli* O157:NM (21). This loss of *stx* genes has important diagnostic implications if laboratories rely on toxin detection to find these organisms late in illness (34). Furthermore, the loss of *stx* complicates epidemiological investigations (11) and might even influence the outcome of the disease (21).

One of integration sites for *stx*$_2$-harboring bacteriophages in the chromosome of SF EHEC O157:NM is the *yecE* gene (11). In this study of the dynamic processes in the evolution of the genome in SF EHEC O157:NM, we compared the integrity of the phage integration site in *stx*$_2$-positive SF EHEC O157:NM strains and their *stx*-negative derivatives and investigated the ability of *stx*$_2$ phages from SF EHEC O157 patients' isolates to lysogenize *stx*-negative variants of SF EHEC O157:NM.

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▽ Published ahead of print on 2 November 2007.

TABLE 1. Molecular and phenotypic traits of consecutive stx_2-positive and *stx*-negative SF *E. coli* O157 isolates from patients A, B, and C

Strain	Molecular trait[a]														Phenotype[b]				ST[c]
	rfb_{O157}	$fliC_{H7}$	stx_2	EHEC *hlyA*	*cdt-V*	*eae*	*efa1*[d]	*sfpA*	$lpfA_{O157\text{-}OI141}$	$lpfA_{O157\text{-}OI154}$	*terE*	*katP*	*espP*	*etpD*	SF/GUD	EHEC-Hly	CDT	Te^r	
A1	+	+	+	+	+	γ	+	+	+	+	−	−	−	+	+/+	−	1:16	−	11
A2	+	+	−	+	+	γ	+	+	+	+	−	−	−	+	+/+	−	1:8	−	11
B1	+	+	+	+	+	γ	+	+	+	+	−	−	−	+	+/+	−	1:8	−	11
B2	+	+	−	+	+	γ	+	+	+	+	−	−	−	+	+/+	−	1:8	−	11
C1	+	+	+	+	+	γ	+	+	+	+	−	−	−	+	+/+	−	1:8	−	11
C2	+	+	−	+	+	γ	+	+	+	+	−	−	−	+	+/+	−	1:4	−	11

[a] The presence of the genes was determined by PCR as described in Materials and Methods; +, presence of the gene; −, absence of the gene.
[b] Phenotypes were determined as described in Materials and Methods. SF, sorbitol fermentation; GUD, production of β-D-glucuronidase; EHEC-Hly, production of EHEC hemolysin; CDT, production of CDT-V (titers determined using Chinese hamster ovary cells); Te^r, tellurite resistance. +, presence of the phenotype; −, absence of the phenotype.
[c] ST, sequence type.
[d] A complete *efa1* gene (ca. 10 kb) was identified in all strains using PCRs targeting the 5′, middle, and 3′ regions of the gene (27).

MATERIALS AND METHODS

Bacterial strains. Three SF EHEC O157:NM strains and their corresponding *stx*-negative derivatives were isolated from stool specimens collected 8 to 11 days after the onset of diarrhea and in subsequent stool specimens from the same patients collected 3 to 8 days later, respectively. All patients had HUS. Pair A1-A2 has been partially described previously (34). Pairs B1-B2 and C1-C2 are from a 15-month-old boy and a 20-month-old girl, respectively. In each pair, isolate 1 is stx_2 positive and isolate 2 is *stx* negative. The stx_2-positive SF EHEC O157:NM strain from the initial stool specimen from patient B (B1; 258/98) has been described previously (11). Strains were isolated as described previously (20, 21, 34). Briefly, stool specimens were inoculated into Hajna broth and grown, and the broth was then enriched for *E. coli* O157 using immunomagnetic separation with Dynabeads coated with anti-*E. coli* O157 antibody (Dynal, Oslo, Norway). Beads with attached *E. coli* O157 were cultured on sorbitol-MacConkey (SMAC) agar (Oxoid, Basingstoke, United Kingdom). Overnight cultures harvested into saline were screened for stx_1, stx_2, rfb_{O157}, and *eae* by PCR (19, 34). SF *E. coli* O157 was isolated from PCR-positive cultures using colony blot hybridization with stx_2 and *eae* probes and slide agglutination with antiserum specific for the *E. coli* O157 lipopolysaccharide (21, 34).

PCR. PCR analyses were performed as described previously (11, 48). stx_2, rfb_{O157}, and *sfpA* (a specific marker for SF *E. coli* O157:NM) (19) were detected using primer pairs LP43 and LP44, O157-F and O157-R, and sfpA-U and sfpA-L, respectively (19, 20); stx_2 genes were subtyped using PCR with primers GK3 and GK4 and restriction of the amplicon with HaeIII (20). Genes encoding other toxins (*cdt-V* and EHEC-*hlyA*) and adhesins (*eae*, *efa1*, $lpf_{O157\text{-}OI141}$, and $lpf_{O157\text{-}OI154}$), other plasmid-carried genes (*katP*, *espP*, and *etpD*), and *terE* (used as a marker for the gene cluster encoding tellurite resistance) (24, 38) were detected using established protocols (9, 12, 26, 27, 49, 51). The *eae* genes were subtyped (56), and the genotype of the *fliC* gene, encoding the structural subunit of the H antigen, was determined as described previously (49, 57). The intact or occupied status of *yecE* was tested using primers EC10 and EC11 (16). The integration of an stx_2-carrying bacteriophage homologous to phage $\Phi 258_{320}$ (originating from SF EHEC O157:NM strain 258/98) (11) in *yecE* in wild-type EHEC strains and lysogens was identified by PCR with primers Int-258_{320} and EC11, which connect the integrase gene of phage $\Phi 258_{320}$ with *yecE* (11).

Multilocus sequence typing. Internal fragments of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were analyzed as described previously (54), except for use of a newly designed forward primer for *icd* (5′-CGA TTATCCCTTACATTGAAG-3′), which yielded more reliable sequencing results. Alleles and sequence types were assigned in accordance with the *E. coli* multilocus sequence typing website (http://web.mpiib-berlin.mpg.de/mlst/dbs/Ecoli).

Induction of stx_2-converting phages from SF EHEC O157:NM and transduction experiments. The stx_2-converting bacteriophage $\Phi 258_{320}$ was induced from SF EHEC O157:NM strain B1 (258/98) (11) using 0.5 μg/ml of mitomycin C (Sigma-Aldrich, Deisenhofen, Germany) (45). One hundred microliters of the phage lysate was mixed with 100 μl of the log-phase culture (10^6 to 10^7 CFU) of an *stx*-negative SF *E. coli* O157:NM strain (A2, B2, or C2) and 125 μl of 0.1 M $CaCl_2$ solution and incubated for 2 h at 37°C without shaking. After that, the mixture was transferred into 4 ml of Luria-Bertani (LB) broth and incubated for 48 h at 37°C and 180 rpm. Tenfold dilutions of the culture were inoculated on LB agar, and overnight growths harvested into 1 ml of saline were screened for stx_2 by PCR with primers LP43 and LP44. The PCR-positive cultures were restreaked on LB agar, and plates containing ~200 well-separated colonies were used to isolate stx_2-containing colonies (putative lysogens) by colony blot hybridization with the stx_2 probe (20, 34). The stx_2-positive colonies were subcultured three times on LB agar and tested by PCR for stx_2 after the third passage to identify stable lysogens (45); the lysogens were checked for the maintenance of stx_2 at 3-month intervals for 1 year. *E. coli* strain C600, which can be lysogenized with phage $\Phi 258_{320}$ (11), was used as a positive control in the transduction experiments.

Phenotypes. Sorbitol fermentation was detected on SMAC after overnight incubation. β-D-Glucuronidase activity was determined on Difco nutrient agar with 4-methylumbelliferyl β-D-glucuronide (Becton Dickinson, Heidelberg, Germany). Production of EHEC hemolysin was detected on enterohemolysin agar (Sifin, Berlin, Germany) and resistance to tellurite on cefixime-tellurite-SMAC (9). Stx production was investigated using a latex agglutination assay (Verotox-F; Denka Seiken Co., Ltd., Tokyo, Japan). Stx cytotoxicity titers in culture supernatants were assessed using the Vero cell assay (30). Production of cytolethal distending toxin V (CDT-V) was tested using the Chinese hamster ovary cell assay (26).

RESULTS

Characteristics of stx_2-positive and *stx*-negative SF *E. coli* O157:NM strains isolated from sequential stool specimens. All three initial EHEC O157:NM strains isolated from stool specimens from the three patients and all three subsequent *stx*-negative isolates shared putative virulence genes encoding non-Stx toxins (EHEC hemolysin and CDT-V) and various adhesins (Table 1). Moreover, all six strains displayed an identical combination of plasmid-carried genes (presence of EHEC-*hlyA*, *etpD*, and *sfpA* and absence of *katP* and *espP*). Each of the strains contained rfb_{O157} and $fliC_{H7}$ but lacked the *terE* gene and, accordingly, failed to grow on cefixime-tellurite-SMAC. All strains fermented sorbitol, produced β-D-glucuronidase, and secreted CDT-V in similar amounts, but all failed to express the enterohemolytic phenotype (Table 1). Moreover, they shared identical sequence types (ST11), indicating identical clonal backgrounds (Table 1). The finding that the stx_2-positive and *stx*-negative intrapatient isolates share *stx*-independent characteristics suggested that the latter organisms descended from the former ones by the loss of stx_2 during infection.

TABLE 2. Presence of stx_2 genes and occupation of *yecE* with an stx_2-harboring phage in SF *E. coli* O157:NM parental stx_2-positive strains, their *stx*-negative derivatives, and lysogens

Patient's isolate or lysogen	Result of PCR targeting:			Stx2 titer (latex agglutination assay)[c]	Stx cytotoxicity titer (Vero cell assay)
	stx_2	*yecE*[a]	*yecE–int*$\Phi258_{320}$[b]		
A1	+	−	+	1:64	1:128
A2	−	+	−	<1:2	<1:2
B1	+	−	+	1:256	1:1,024
B2	−	+	−	<1:2	<1:2
C1	+	−	+	1:128	1:256
C2	−	+	−	<1:2	<1:2
B2($\Phi258_{320}$)-1	+	−	+	1:64	1:64
B2($\Phi258_{320}$)-2	+	−	+	1:64	1:128
B2($\Phi258_{320}$)-3	+	−	+	1:128	1:256
B2($\Phi258_{320}$)-4	+	−	+	1:128	1:128
C2($\Phi258_{320}$)-1	+	−	+	1:128	1:256
C2($\Phi258_{320}$)-2	+	−	+	1:64	1:128
C600	−	+	−	<1:2	<1:2
C600($\Phi258_{320}$)	+	−	+	1:16	1:32

[a] +, the locus is intact; −, the locus is occupied by foreign DNA.
[b] +, a phage with the *int* gene homologous to that of phage $\Phi258_{320}$ is integrated in *yecE*; −, the phage is absent.
[c] Verotox-F (Denka Seikesn, Tokyo, Japan).

Integrity of *yecE* in stx_2-positive and *stx*-negative SF *E. coli* O157:NM strains. *yecE* was occupied in each of the stx_2-positive strains of the original infection (A1, B1, and C1) (Table 2; Fig. 1, lanes 1 to 3) and was intact in all three *stx*-negative strains (A2, B2, and C2) (Table 2; Fig. 1, lanes 4 to 6).

Transduction of *stx*-negative SF *E. coli* O157:NM with an stx_2-harboring phage from SF EHEC O157:NM. A stable (for at least 12 months) infection with phage $\Phi258_{320}$ could be achieved in two of the three *stx*-negative SF *E. coli* O157:NM strains. The remaining strain (A2) could be transiently infected but lost the phage after the second passage on LB agar. Four stable lysogens (among 246 transduced colonies) originated from the cognate *stx*-negative strain B2, an *stx*-negative derivative of strain B1 (258/98) (transduction frequency of 1.6%) and were designated B2($\Phi258_{320}$)-1 to B2($\Phi258_{320}$)-4. Two stable lysogens (among 221 transduced colonies) originated from a noncognate *stx*-negative strain (C2) derived from EHEC strain C1 (transduction frequency of 0.9%) and were designated C2($\Phi258_{320}$)-1 and C2($\Phi258_{320}$)-2. The lysogenization of the *stx*-negative strains with stx_2-harboring phage $\Phi258_{320}$ resulted in the acquisition of stx_2 by each of the lysogens, as demonstrated by PCR with primers LP43-LP44 (Table 2; Fig. 1, lanes 7 to 12). Moreover, phage $\Phi258_{320}$ also transduced the stx_2 gene into the control strain *E. coli* C600 (Table 2; Fig. 1, lane 14).

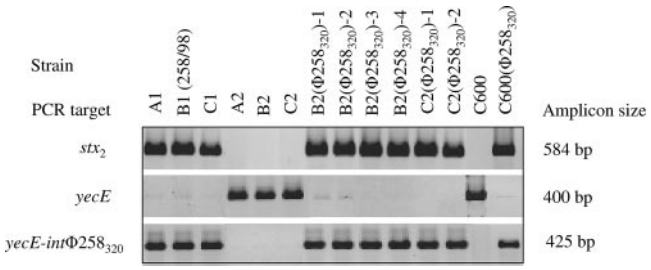

FIG. 1. PCR analysis of occupation of *yec*E in SF EHEC O157:NM clinical isolates, their *stx*-negative derivatives, and lysogens. The strains tested, PCR targets, and lengths of PCR amplicons are listed across the top and to the left and right of the rows of amplicons, respectively. Strain B2 (258/98), harboring phage $\Phi258_{320}$ integrated in *yecE* (11), and *E. coli* K-12 C600, which has *yecE* intact (13), were used as controls. In PCR targeting *yecE*, the presence of an amplicon indicates that the locus is intact, whereas the absence of an amplicon (or a very weak amplicon) indicates that the locus is occupied by foreign DNA. In PCR connecting *yecE* with the integrase gene (*int*) of phage $\Phi258_{320}$, the presence of an amplicon indicates that a phage with a homologous *int* gene is integrated in *yecE*; the absence of an amplicon indicates the absence of such phage.

Stx production. The latex agglutination assay confirmed that all three parental stx_2-containing SF EHEC O157:NM strains produced Stx2 (titers of 1:64 to 1:256) and that their *stx*-negative derivatives did not (titers of <1:2) (Table 2). Each of the six lysogens produced Stx2 in titers (1:64 to 1:128) comparable to that of the donor strain B1 (1:256) (Table 2). Stx2 produced by the lysogens was cytotoxic toward Vero cells (Table 2), indicating that the biological activity is similar to that of Stx2 produced by the wild-type SF EHEC O157:NM strains.

Phage integration sites. In each lysogen, the acquisition of the stx_2-harboring phage $\Phi258_{320}$ resulted in the occupation of *yecE* (Table 2; Fig. 1, lanes 7 to 12), which had been intact in the *stx*-negative recipient strains B2 and C2 (Table 2; Fig. 1, lanes 5 and 6, respectively). We confirmed that *yecE* in the lysogens was occupied by phage $\Phi258_{320}$, using PCR to link *yecE* with the integrase gene of phage $\Phi258_{320}$. The PCR produced an amplicon of 425 bp in strain B1 (258/98) (and also A1 and C1) (Fig. 1, lanes 1 to 3) and each of the lysogens (Fig. 1, lanes 7 to 12), demonstrating that all these strains contained a phage homologous to phage $\Phi258_{320}$ integrated in *yecE*. As expected, phage $\Phi258_{320}$ also integrated into *yecE* in the con-

trol *E. coli* strain C600 (Fig. 1, lane 14). Taken together, these data demonstrate that *stx*-negative SF *E. coli* O157:NM strains can be converted to EHEC O157:NM via transduction with an stx_2-harboring phage originating from an EHEC O157:NM strain and that *yecE* plays an essential role in this process.

DISCUSSION

One noteworthy aspect of EHEC infections is that the infecting organism can undergo major genotype and phenotype changes during the course of infection in humans (10, 21, 34). We have demonstrated interconversion between various pathotypes of SF *E. coli* O157:NM, in which EHEC O157:NM strains harboring stx_2 lose stx_2 and *stx*-negative *E. coli* O157:NM can be transduced by an stx_2-harboring phage from an EHEC O157 patient's isolate. In these events, *yecE* is a hot spot for integration and excision of stx_2 phages. The ability to undergo bidirectional conversion from one pathotype to another enables recycling of an important pathogenic trait in SF *E. coli* O157:NM and indicates that these organisms can respond to environmental influences with a high degree of flexibility and that this property increases their capacity to survive under a variety of conditions in the human host and reservoir(s).

Although *yecE* is only rarely occupied by phages in EHEC O157:H7 (16, 46), our analysis of consecutive stx_2-positive and *stx*-negative SF *E. coli* O157:NM strains confirms our previous observation that *yecE* is an important integration site for Stx2-encoding phages in SF EHEC O157:NM strains (11). It is of interest that up to now no other function is known for the *yecE* gene or its product. *yecE* in all stx_2-positive SF EHEC O157:NM strains was occupied by an stx_2-converting phage, whereas in all *stx*-negative SF *E. coli* O157:NM strains the *yecE* genes were intact (Fig. 1). Lysogenization of *stx*-negative strains with stx_2-containing phage $\Phi 258_{320}$ originating from a SF EHEC O157:NM clinical isolate resulted in the occupation of *yecE* in the lysogens (Fig. 1). The presence of phage $\Phi 258_{320}$ in *yecE* in the genomes of the lysogens could be confirmed by the signal elicited from all lysogens in the PCR connecting *yecE* with the integrase gene of phage $\Phi 258_{320}$ (Fig. 1). Together with acquiring the stx_2 gene, all lysogens also acquired the ability to produce Stx2 cytotoxic to Vero cells. This demonstrates that via transduction with the stx_2 phage, the *stx*-negative, *eae*-positive SF *E. coli* O157:NM strains were converted to biologically potent EHEC. In contrast to the case for SF EHEC O157:NM, stx_2 phages in EHEC O157:H7, including both sequenced strains EDL933 and Sakai (24, 32, 38, 40) and other clinical isolates (6, 47), use *wrbA* as a common genomic integration site, whereas *yehV* is frequently used for integration of stx_2 phages in EHEC O157:H7 isolated from cattle (46). In addition, other, mostly yet-unidentified chromosomal loci have been proposed to serve as integration sites for stx_2-harboring phages in the genome of EHEC O157:H7 (6, 36, 47). Although it is presently unknown why *yecE*, which is intact in *E. coli* O157:H7 strains EDL933 and Sakai (24, 38) and other isolates (unpublished data), does not regularly serve as an stx_2 phage integration site in these organisms (46), this fact is in accordance with a recent observation that the selection of an *stx* phage integration site preferentially depends on the host strain rather than on the phage (46). The greater stability of stx_2 in EHEC O157:H7 than in SF EHEC O157:NM (21, 34) might be attributable to the difference in the integration sites for stx_2-harboring phages in the two organisms.

The observation that *stx*-negative SF *E. coli* O157:NM strains occur more frequently than *stx*-positive SF *E. coli* O157:NM strains in the gastrointestinal tracts of animals (31, 43, 59) suggests that an *stx*-negative phase might be a normal occurrence in the life cycle of SF EHEC O157:NM in the environment. The *stx*-negative condition might enable the pathogens to avoid lysis triggered by various phage-inducing stimuli, such as H_2O_2 (52), thereby conferring a selective advantage to survive under different conditions. If analogous to *E. coli* O157:H7 (15), the absence of an *stx* phage would not influence the ability of *stx*-negative SF *E. coli* O157 organisms to colonize the intestines of animals and thus to be spread in the environment via fecal contamination. We propose that such *stx*-negative, *eae*-positive SF *E. coli* O157:NM strains, which can be converted to EHEC by transduction with stx_2 phages from EHEC O157 in vitro, represent "EHEC wannabes." The acquisition of an *stx* phage by such organisms not only makes them highly pathogenic for the human host but also could play a role in their ecology. It has been recently shown that the carriage of an Stx-encoding prophage increases the rate of survival of *E. coli* O157:H7 within grazing protozoa (*Tetrahymena pyriformis*) (50) and thus in the environment. Apparently, both gain and loss of *stx* phages can contribute to the evolution of SF *E. coli* O157:NM. Because of their ability to recycle stx_2, which is the major virulence trait, SF *E. coli* O157:NM can be considered ephemeral EHEC strains that can exist as *stx*-negative organisms during certain phases of their life cycle.

A change in the pathotype of the infecting strain during infection has consequences regarding the laboratory diagnosis and epidemiological investigations. Loss of the *stx* gene makes it impossible to correctly identify *stx*-negative variants using methods based on the detection of only *stx* and/or Stx. To overcome this limitation, we recommend the parallel use of procedures independent of the presence of *stx*, in particular the detection of *eae* using PCR (34). In the case of SF *E. coli* O157:NM, detection of O157 lipopolysaccharide antigen by slide agglutination and/or its encoding gene using PCR and, especially, detection of the *sfpA* gene, which is a unique marker for SF *E. coli* O157:NM (19), allow identification of both *stx*-positive and *stx*-negative strains (19). Furthermore, loss of *stx* genes in strains that are related epidemiologically can change the pulsed-field gel electrophoresis pattern (11), thereby complicating epidemiological investigations of SF *E. coli* O157:NM disease (11). Therefore, the possibility of an altered pulsed-field gel electrophoresis pattern arising from the loss of *stx* should be always considered when interpreting the DNA fingerprints of potentially epidemiologically related SF *E. coli* O157:NM strains.

Ongoing studies in our laboratories aim to investigate the conditions favoring the loss of *stx* and the possibility of promoting the excision of *stx* and conversion to less pathogenic *stx*-negative EHEC. In the absence of specific anti-EHEC treatment, such an intervention would offer a new and powerful preventive and therapeutic approach.

ACKNOWLEDGMENTS

This study was supported by a grant from the EU Network ERA-NET PathoGenoMics (project number 0313937C), by a grant from the EU Network of Excellence EuroPathoGenomics (number LSHB-CT-2005-512061), by a grant from the Interdisciplinary Center of Clinical Research (IZKF) Münster (project no. Ka2/061/04), and by 973 Program grant 2005CB522904 from the Ministry of Science and Technology, People's Republic of China.

We thank Phillip I. Tarr (Washington University School of Medicine, St. Louis, MO) for helpful discussion during preparation of the manuscript.

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