INFECTION AND IMMUNITY, Mar. 2000, p. 1400–1407 Vol. 68, No. 3
0019-9567/00/$04.00+0


# Iha: a Novel *Escherichia coli* O157:H7 Adherence-Conferring Molecule Encoded on a Recently Acquired Chromosomal Island of Conserved Structure

PHILLIP I. TARR,[1,2,3]* SIMA S. BILGE,[1,2] JAMES C. VARY, JR.,[1] SRDJAN JELACIC,[1,2] REBECCA L. HABEEB,[1] TERESA R. WARD,[1] MICHAEL R. BAYLOR,[1] AND THOMAS E. BESSER[4]

*Division of Gastroenterology, Children's Hospital and Regional Medical Center,[1] and Departments of Pediatrics[2] and Microbiology,[3] University of Washington School of Medicine, Seattle, and the Department of Veterinary Microbiology and Pathology, Washington State University College of Veterinary Medicine, Pullman,[4] Washington*

Received 10 September 1999/Returned for modification 10 November 1999/Accepted 23 November 1999

**The mechanisms used by Shiga toxin (Stx)-producing *Escherichia coli* to adhere to epithelial cells are incompletely understood. Two cosmids from an *E. coli* O157:H7 DNA library contain an adherence-conferring chromosomal gene encoding a protein similar to iron-regulated gene A (IrgA) of *Vibrio cholerae* (M. B. Goldberg, S. A. Boyko, J. R. Butterton, J. A. Stoebner, S. M. Payne, and S. B. Calderwood, Mol. Microbiol. 6:2407–2418, 1992). We have termed the product of this gene the IrgA homologue adhesin (Iha), which is encoded by *iha*. Iha is 67 kDa in *E. coli* O157:H7 and 78 kDa in laboratory *E. coli* and is structurally unlike other known adhesins. DNA adjacent to *iha* contains tellurite resistance loci and is conserved in structure in distantly related pathogenic *E. coli*, but it is absent from nontoxigenic *E. coli* O55:H7, sorbitol-fermenting Stx-producing *E. coli* O157:H−, and laboratory *E. coli*. We have termed this region the tellurite resistance- and adherence-conferring island. We conclude that Iha is a novel bacterial adherence-conferring protein and is contained within an *E. coli* chromosomal island of conserved structure. Pathogenic *E. coli* O157:H7 has only recently acquired this island.**

*Escherichia coli* O157:H7 and other Shiga toxin (Stx)-producing *E. coli* (STEC) strains cause diarrhea, hemorrhagic colitis, and the hemolytic uremic syndrome. The mechanisms underlying the adherence of STEC to epithelial cells are only partly understood (35). The ability to adhere to epithelial cells is an important virulence trait, because adherence presumably enables enteric pathogens to deliver toxins efficiently to host organs, overcome peristaltic clearance, and gain access to host-derived nutrients.

Intimin is the best-characterized *E. coli* O157:H7 adherence molecule. Encoded by *eae*, intimin mediates the attaching and effacing lesion caused by enteropathogenic *E. coli* (EPEC) and many STEC serotypes (21) and is an important component of pathogenicity. However, cloned *eae* from EPEC and STEC do not confer the adherent phenotype upon laboratory *E. coli* (18, 25, 28). Moreover, though the cloned EPEC locus of enterocyte effacement, which includes *eae*, does confer the adherence phenotype on *E. coli* K-12 (27), the cloned *E. coli* O157:H7 locus of enterocyte effacement does not (12).

We describe an *E. coli* O157:H7 gene that renders laboratory *E. coli* adherent to epithelial cells and explore evolutionary aspects of its acquisition.

(These data were presented in part at the 3rd International Symposium and Workshop on Shiga Toxin-Producing *Escherichia coli* Infections, Baltimore, Md., 22 to 26 June 1997.)

## MATERIALS AND METHODS

**Bacteria.** The bacteria analyzed in this study are described in Table 1. The bacteria were inoculated directly from frozen stock (in Luria-Bertani [LB] broth–15% glycerol, maintained at −70°C) into LB broth (26). The cultures were grown overnight under standardized conditions (37°C; 14 to 16 h; stationary cultures) for adherence assays and protein preparations. The bacteria were grown in a shaking incubator (37°C; 14 to 24 h) for DNA preparations or matings. Ampicillin (200 mg/liter), nalidixic acid (20 mg/liter), or both were added if appropriate. Unless otherwise specified, *E. coli* O157:H7 strain 86-24 (37) and its DNA were used.

**Adherence assay.** HeLa or Madin-Darby bovine kidney (MDBK) cells were grown to confluence at 37°C in 5% $CO_2$ in plastic flasks in minimal essential medium with 10% (vol/vol) heat-inactivated fetal calf serum, 2 mM L-glutamine, penicillin (100,000 IU/liter), and streptomycin (100 mg/liter). The cells were then trypsinized, diluted, added to four-chamber glass slides (Nunc, Naperville, Ill.), and reincubated. Two days later, the chambers were washed with sterile phosphate-buffered saline (PBS) and replenished with 0.6 ml of incubation medium (minimal essential medium, fetal calf serum [5% for HeLa cells or 10% for MDBK cells], 2 mM L-glutamine, nonessential amino acids, 0.5% D-mannose, and ampicillin [200 mg/liter] if appropriate). Twenty microliters of overnight LB broth cultures of bacteria, including positive (*E. coli* B171) and negative (*E. coli* ORN172) controls, was added to individual chambers. The slides were incubated (3 h; 37°C in 5% $CO_2$), washed three times with PBS, covered with incubation medium (0.6 ml), incubated (2 h; 37°C in 5% $CO_2$), washed 10 times with PBS, fixed (100% methanol; 5 min), Giemsa stained (60 min), and coverslipped.

A microscopist unaware of the identity of the bacteria being examined counted the total number of clusters (≥5 bacteria/cluster), bacteria, and cells in five separate fields in each chamber enumerated. Adherence indices (clusters per cell, bacteria per cluster, and bacteria per cell) were calculated. Comparisons were made between pairs of chambers assayed on the same day. The significance of differences in adherence indices was determined using the two-tailed Student *t* test or the Mann-Whitney rank sum test if tests for normal distribution and equal variances were passed or failed, respectively (14).

**DNA preparation.** For cosmid cloning, DNA was prepared from bacteria lysed in ultracentrifuge tubes (9). For PCR amplifications and for Southern blotting, DNA was prepared from bacteria suspended in 50 mM Tris-HCl (pH 8.0) with 50 mM EDTA, to which sodium dodecyl sulfate (SDS) and proteinase K (Sigma, St. Louis, Mo.) (final concentrations, 1 and 0.04%, respectively) were added. Following incubation of the bacteria with SDS and proteinase K (65°C; 2 h), DNA was extracted (with phenol-chloroform) and precipitated (with ammonium acetate-ethanol). Plasmids were purified by CsCl density gradient centrifugation or alkaline lysis (26).

**Cosmid library.** *Xba*I-digested plasmid Supercos (pSC) (Stratagene, La Jolla, Calif.) was treated with calf intestinal alkaline phosphatase (Boehringer Mannheim, Indianapolis, Ind.), phenol extracted, and digested with *Bam*HI. The cosmid arms were ligated to a calf intestinal alkaline phosphatase-treated *Sau*3A partial digest of *E. coli* O157:H7 DNA, packaged with the Gigapack II XL system (Stratagene), transduced into *E. coli* NM554 (38), and tested for adherence.

* Corresponding author. Mailing address: MS: CH-24, Children's Hospital and Regional Medical Center, 4800 Sand Point Way NE, Seattle, WA 98105. Phone: (206) 526-2521. Fax: (206) 528-2721. E-mail: tarr@u.washington.edu.

TABLE 1. Bacteria analyzed in this study

| Strain(s) | Reference or source | Homology | | |
|---|---|---|---|---|
| | | *iha*[b] | TAI[c] | Iha[d] |
| *E. coli* O157:H7[a] | | | | |
| 25 *E. coli* O157:H7 strains from Seattle, Australia, and Colombia, including strain 86-24 | 37, 5; M. Samadpour, S. Mattar, Australia Government Analytical Laboratories | 4.7, 7.5[e] | + | 67 |
| *E. coli* O157:H7 (86-24$^{nalR}$) | 6 | 4.7 | + | NT |
| *E. coli* O157:H7 (86-24$^{nalR}$) (Δ*iha*) | This paper | 2.7[f] | + | ± |
| Sorbitol-fermenting *E. coli* O157:H− (493-89, 5412-89, CB569, 514-91) | 22, 43; H. Karch, L. Beutin | | − | NT |
| *E. coli* O55:H7 (5A-D, TB156A, TB182A) | 41, 7 | | − | NT |
| *E. coli* O55:H7 (5E) | 41 | 2.9 | V | − |
| *eae*$^+$ STEC distantly related to *E. coli* O157:H7 | | | | |
| *E. coli* O26:NM (TB352A) | 7 | 4.7, 10.1 | + | 39 |
| *E. coli* O85:NM (TB334C) | 7 | 4.7, 10.1 | + | − |
| *E. coli* O126:H2 (TB285A) | 7 | 4.7, 10.1 | + | − |
| *E. coli* O103:H2 (UTI) | 36 | 4.7 | + | − |
| *E. coli* O111:HN (TB226A) | 7 | 4.7 | + | − |
| *E. coli* O103:H6 (TB154A) | 7 | | − | NT |
| *eae*-negative STEC distantly related to *E. coli* O157:H7 | | | | |
| *E. coli* O104:H21 | 3; T. Damrow | 3.9, 10.1 | V | 56 |
| *E. coli* O113:H21 (CL15) | 11 | 7.9 | V | 56 |
| *stx*-negative EPEC distantly related to *E. coli* O157:H7 | | | | |
| *E. coli* O15:NM (RDEC-1) | 8 | 4.7 | + | − |
| *E. coli* O111:NM (B171) | 32 | | − | NT |
| *E. coli* O119:H6 (659-79) | 23 | | − | NT |
| *E. coli* O127:H6 (E2348/69) | 23 | | − | NT |
| *E. coli* O142:H6 (E851/71) | 23 | | − | NT |
| *E. coli* O111:H− (2430-78) | 23 | 5.1 | + | − |
| Other | | | | |
| *E. coli* ORN172(pSK+) | 42, this paper | | − | − |
| *E. coli* ORN172(pIHA) | 42, this paper | 2.2[g] | − | 78 |
| 20 *E. coli* strains from nondiarrheal human stools | S. Moseley | 3.6[h] | NT | NT |

[a] Including derivatives and closely related strains.
[b] Size (in kilobases) of *Bst*XI fragment(s) detected by *iha* probe (the insert of pIha). −, *iha* homologue was not detected with this probe.
[c] Characterization of *Bst*XI fragments detected by TAI probe. +, TAI homologue detected. −, TAI probe detects *Bst*XI fragments that are of different sizes, and fewer, than those detected in *E. coli* O157:H7; homology is considerably less intense, and the *iha* homologue is absent. V, one or more *Bst*XI framgents are intense on a Southern blot using the TAI probe, but the pattern of bands detected by the TAI probe is different from that of TAI in *E. coli* O157:H7; *iha* homologue(s) is present. NT, not tested.
[d] Size (in kilodaltons) of the largest OMP detected by α-Iha antibodies. NT, not tested. −, antigen not detected; ±, faint 67-kDa antigen detected in some OMP preparations.
[e] In five *E. coli* O157:H7 strains, a 7.5-kb *Bst*XI fragment is also detected by the *iha* probe.
[f] Faint 2.7-kb band detected, representing homology of *iha* probe to intact 5′ and 3′ ends of truncated *iha* gene.
[g] *iha* is cloned into pSK+ in this laboratory strain. Size reflects insert length, not *Bst*XI fragment.
[h] Only three of these isolates contain *iha* homologues.

**Southern hybridization.** Two micrograms of *Bst*XI-digested bacterial DNA was electrophoresed in 1% agarose–0.5× Tris-borate-EDTA, stained with ethidium bromide, transferred to a nylon membrane (Micron Separations, Westboro, Mass.), and UV cross-linked. The membranes were then immersed in hybridization buffer (10% polyethylene glycol, 7% SDS, and 1.5× SSPE [1× SSPE is 0.18 M NaCl, 10 mM $NaH_2PO_4$, and 1 mM EDTA, pH 7.7]) (26) (2 h; 65°C). Probes for *eae*, the large *E. coli* O157:H7 plasmid (pO157), and *stx2* were derived from pCVD434 (18), pCVD419 (24), and pNN111-19 (30), respectively. Probes were also derived from the inserts of pIha, pSC(A-G6), and pSC(T-H12), described below. The fragments were labeled with the Megaprime DNA system (Amersham, Arlington Heights, Ill.) and [α-$^{32}$P]dATP (New England Nuclear Research Products, Boston, Mass.) and added to the membranes. The membranes and probes were incubated overnight (65°C), washed twice (15 min; 65°C) in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (26)–0.1% SDS and twice (15 min; 65°C) in 0.2× SSC–0.1% SDS and exposed to X-ray film in the presence of intensifying screens (−70°C).

**Gene identification and sequencing strategy.** pSC(A-G6) segments were cloned into pSK+ and tested for their abilities to confer adherence on laboratory *E. coli*. An 8,040 bp *Kpn*I adherence-conferring fragment was sequenced in both directions using the *Taq* DyeDeoxy cycle-sequencing kit and a model 373A DNA sequencer (Applied Biosystems, Foster City, Calif.). The sequences were compared to the National Center for Biotechnology Information Geninfo BLAST network server database (13). A 2,088-bp adherence-conferring open reading frame (ORF) was amplified using PCR and the primers 5′GGGGATCCAATT **CTGGCATGCCGAGGCAGTGC**3′ and 5′GGTCTAGATTCT**CGTTGCCACTG TTCCGCCAGG**3′ (the boldface nucleotides represent sequences derived from the 8,040-bp *Kpn*I adherence-conferring fragment). These primers contain 5′ *Bam*HI and *Xba*I sites, respectively. The primers produce an amplicon which includes the ORF, as well as 141 bp 5′ to its ATG start codon and 80 bp 3′ to its TGA stop codon. The amplicon was digested with *Bam*HI and *Xba*I and cloned into corresponding sites in pSK+, resulting in a construct designated pIha.

**Deletion mutant.** An in-frame deletion of the 2,088-bp ORF of interest was created in *E. coli* O157:H7$^{nalR}$ by PCR using the primer pairs 5′GGAGCGAG CTC**GCCTTATCACGACTACGAATACCAGC**3′ (primer A)-5′GGTAGGATC**C CTCTGCAGCAGCTATGCTGCTGGC**3′ and 5′GCTAGGATC**CCAGACGGGA TCATCAACAACAGGA**3′-5′GCTATCTCGAG**GGGACTTATCTGACCGGGCC CCTGG**3′ (primer B) and *E. coli* O157:H7 DNA. These primers contain 5′ *Sac*I, *Bam*HI, *Bam*HI, and *Xho*I sites, respectively; the boldface nucleotides were derived from the sequence of the 8,040-bp *Kpn*I adherence-conferring fragment. The resulting 698- and 558-bp amplicons span the 5′ and 3′ ends of the gene of interest. These PCR products were digested with *Bam*HI and then with *Sac*I or *Xho*I, respectively, and separately cloned into pSK+. *Sac*I-*Bam*HI and *Bam*HI-

*Xho*I inserts were excised, purified, ligated to each other at their *Bam*HI sites, and cloned into *Sac*I-*Xho*I-digested pSK+, resulting in pSK+(Δ*iha*). The insert of pSK+(Δ*iha*) was excised and ligated into pCVD442 (10). The resulting pCVD442(Δ*iha*) was transformed into *E. coli* SM10(λpir) (34).

*E. coli* SM10(λpir)(pCVD442(Δ*iha*)) and *E. coli* O157:H7$^{nalR}$ were mated on LB agar at 37°C, and a transconjugant was selected by plating the mated bacteria on LB agar with ampicillin and nalidixic acid. This presumed merodiploid was then grown overnight (37°C) in LB broth without salt, plated onto LB agar containing 5% sucrose but no salt, and incubated (30°C). DNA from the resulting sucrose-resistant, ampicillin-sensitive, O157 antigen-expressing (confirmed with a latex particle agglutination test [Oxoid, Basingstoke, Hampshire, United Kingdom]) putative deletion mutant [designated *E. coli* O157:H7$^{nalR}$(Δ*iha*)] was analyzed on Southern blots and PCR amplified with primers A and B. The resulting amplicon was cloned into pSK+ and sequenced, to confirm that the 647 amino acids between Glu$^{25}$ and Gln$^{673}$ were replaced by a Gly and Ser, as intended.

**Antibodies.** Polyclonal antibodies were raised in rabbits immunized with (C)YTWTRSEQRDGDNKG-COOH coupled to keyhole limpet hemocyanin via the Cys residue using the PolyQuik protocol (Zymed Laboratories, South San Francisco, Calif.). Affinity-purified antibodies to the Iha peptide (α-Iha antibodies) were produced by Zymed using a peptide-conjugated affinity matrix.

**OMP and total bacterial protein (TBP) analysis.** Outer membrane proteins (OMPs) were prepared (1) from bacteria grown overnight (37°C in 100 ml of LB) to ascertain if the protein of interest localized to the cell envelope. Protein concentrations were determined with the Protein Assay Kit (Bio-Rad, Hercules, Calif.). For proteinase K susceptibility experiments (performed to establish if the molecule of interest possessed externally directed domains), two 100-ml cultures of bacteria were pooled, pelleted, washed once with 10 mM Tris-HCl (pH 8.0) (Tris), suspended in 2 ml of Tris with 1 mM EDTA, and divided into two equal aliquots. Twenty-five microliters of proteinase K (20 mg/ml) or water was added to each aliquot, and the tubes were then shaken (37°C; 1 h). Eight microliters of phenylmethylsulfonylfluoride (0.2 M) was then added to the samples, which were again shaken (37°C; 15 min), pelleted, washed twice (in Tris with 5 mM $MgCl_2$), and suspended in 100 μl of Tris. Fifty microliters of suspended bacteria was added to 10 ml of Tris for OMP preparation. Triton-X (11.1 μl; 0.01%) in water was added to the remaining 50 μl, which was boiled (20 min) and iced. Then 12 μl of 10× DNase buffer and 2 μl of DNase (1 U/μl) (Promega, Madison, Wis.) were added to the tubes, which were incubated at room temperature (30 min). Then 5× loading buffer (32 μl) was added to each tube, and samples were stored at −20°C until they were studied.

OMPs in loading buffer (1 μg/lane) or TBPs equivalent to the bacteria in 35 μl [*E. coli* O157:H7 and *E. coli* O157:H7$^{nalR}$(Δ*iha*)] or 30 ml [*E. coli* ORN172(pSK+) and *E. coli* ORN172(pIha)] of overnight broth culture were separated in SDS–10% polyacrylamide gel electrophoresis gels and transferred to polyvinylidene fluoride membranes (Immobilon-P; Millipore, Bedford, Mass.) or Coomassie stained. The different volumes of TBP loaded reflect different levels of expression of maltose-binding protein (Mbp) observed in preliminary experiments (data not shown).

The membranes were blocked overnight at 4°C in antibody buffer (PBS with 0.05% [vol/vol] Tween 20) containing 5% nonfat dried milk and 0.02% sodium azide. The membranes were washed once and incubated overnight with α-Iha antibodies diluted 1:2,000 or with affinity-purified antibodies to Mbp (α-Mbp antibodies) (New England Biolabs, Beverly, Mass.) diluted 1:50,000 in antibody buffer. The blots were then washed three times in antibody buffer, incubated for 30 min with affinity-purified goat anti-rabbit immunoglobulin G (H+L) peroxidase conjugate (Boehringer Mannheim) diluted 1:2,000 in antibody buffer, and washed three times in antibody buffer. After the blocking, all washes and incubations were performed at room temperature. Bound antibodies were detected with SuperSignal chemiluminescent substrate, Western blotting (Pierce, Rockford, Ill.).

**Nucleotide sequence accession number.** The sequence of the 8,040-bp *Kpn*I adherence-conferring fragment has been entered into GenBank as submission AF126104.

## RESULTS

**Characterization of adherence-conferring cosmids and gene.** Two [pSC(A-G6) and pSC(T-H12)] of 2,200 cosmids constructed from *E. coli* O157:H7 DNA mediated the diffuse adherence to HeLa cells of transduced *E. coli* NM554. pSC(A-G6) and pSC(T-H12) each contain ca. 35 kbp of chromosomal DNA, and they overlap by ca. 15 kbp. Southern hybridization determined that neither cosmid contains *eae* or *stx2*. An 8,040-bp *Kpn*I fragment from the overlap region of pSC(A-G6) cloned into pSK+ confers upon *E. coli* ORN172 the ability to adhere diffusely to epithelial cells (see below). This 8,040-bp *Kpn*I fragment is present in *E. coli* O157:H7 (data not shown) DNA and is therefore not an artifact of cosmid construction.

The 8,040-bp *Kpn*I fragment (GenBank number AF126104) has a 45% G+C content and contains five ORFs of interest. Four ORFs are homologous to tellurite resistance genes of *Alcaligenes* sp. (19) and *Serratia marcescens* and are designated *tlpA* to *-D* (for tellurite resistance proteins). A fifth ORF of interest is 2,088 bp long and encodes a protein with a deduced mass of 76,494 da. An amplicon of this 2,088-bp ORF, when cloned into pSK+ (resulting in a construct designated pIha, described below) and transformed into *E. coli* ORN172, conferred upon that laboratory strain the ability to adhere diffusely to epithelial cells. In contrast, *E. coli* ORN172 that had been transformed with pSK+ did not adhere; the respective median (range) bacteria per cell were 4.9 (0.8 to 5.4) and 0.03 (0.00 to 0.06), $P = 0.029$ (Fig. 1A and B). *E. coli* ORN172(pIha) also adhered to MDBK cells (Fig. 1C and D).

This 2,088-bp adherence-conferring ORF from *E. coli* O157:H7 has a 52% G+C content. Of the amino acids this gene encodes, 53% can be matched exactly or conservatively (2) to amino acids in IrgA of *Vibrio cholerae*, encoded by iron-regulated gene A (*irgA*) (15) (Fig. 2). Six of seven amino acids composing a putative TonB box in IrgA can be identically or conservatively matched to amino acids in a potential TonB box in the *E. coli* O157:H7 IrgA homologue. Because of its similarity to this *V. cholerae* protein, we have designated the *E. coli* O157:H7 adherence-conferring protein the IrgA homologue adhesin (Iha), encoded by *iha*.

*E. coli* O157:H7 *iha* lacks an upstream *irgB* homologue and a ferric uptake and regulation protein-binding site, unlike *irgA* in *V. cholerae*. A putative Shine-Dalgarno sequence (GGAG) is located 9 nucleotides upstream of the *iha* start codon. Of the 2,635 contiguous nucleotides starting at the 34th nucleotide from the A of the initiation codon of the *irgA* homologue of *E. coli* O157:H7, 99% are identical to ORF R4 (described as a putative exogenous ferric siderophore receptor) in a pathogenicity-associated island (PAI) of pyelonephritogenic *E. coli* strain CFT073 (20). Homology between the 5′ ends of the *irgA* homologue of *E. coli* O157:H7 and of ORF R4 of *E. coli* strain CFTO73 cannot be further assessed without additional sequence of this *E. coli* CFT073 PAI.

To determine if *E. coli* O157:H7 requires Iha to adhere to epithelial cells, we compared adherence indices for *E. coli* O157:H7$^{nalR}$ and its derivative, *E. coli* O157:H7$^{nalR}$(Δ*iha*). *E. coli* O157:H7$^{nalR}$(Δ*iha*) has sustained an in-frame deletion of 1,941 bp in *iha*, corresponding to the replacement of 647 amino acids with a Gly and a Ser. *E. coli* O157:H7$^{nalR}$(Δ*iha*) adheres to HeLa cells less well than does *E. coli* O157:H7$^{nalR}$ ($0.13 \pm 0.10$ [standard deviation] clusters/cell and $8.8 \pm 1.5$ bacteria/cluster versus $0.25 \pm 0.16$ clusters/cell and $11.4 \pm 4.3$ bacteria/cluster), but not significantly so (respective $t_{8df}$ values, 1.306 and 1.391; respective $P$ values, 0.23 and 0.20). In contrast, pSK+(Δ*iha*), from which the same nucleotides in *iha* have been deleted, does not confer adherence on *E. coli* ORN172.

**Conservation of *iha* and of surrounding DNA.** Twenty-five of 25 *E. coli* O157:H7 strains of diverse origins, 5 of 6 *eae*$^+$ non-O157:H7 STEC strains isolated from patients in Seattle, and RDEC-1, a rabbit EPEC strain that probably evolved from an STEC progenitor (40), each contains a 4.7-kb *Bst*XI DNA fragment that is homologous to the probe consisting of the insert of pIha (Table 1 and Fig. 3). Three non-O157:H7, *eae*$^+$ STEC strains possess a second *Bst*XI fragment of approximately 10.1 kb that is also detected by this *iha* probe (Fig. 3). Five of 25 *E. coli* O157:H7 strains also have a second *Bst*XI fragment of approximately 7.5 kb that hybridizes to the *iha* probe (data not shown). *eae*-negative STEC O104:H21 and O113:H21 contain DNA that is homologous to the *iha* probe on 3.9- and 10.1-kb and 7.9-kb *Bst*XI fragments, respectively

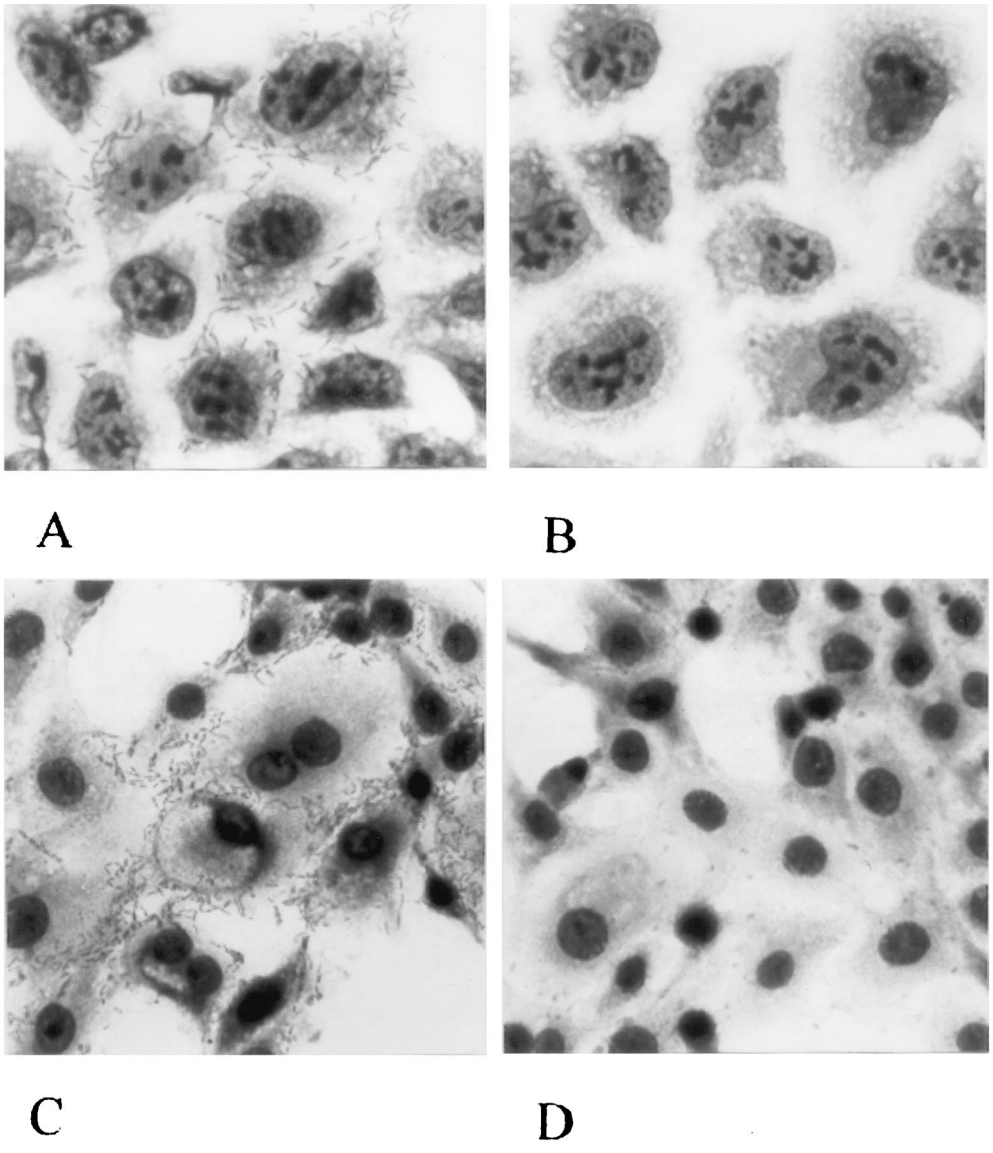


FIG. 1. Adherence of recombinants and controls to epithelial cells. HeLa (A and B) (×600) or MDBK (C and D) (×480) cells incubated with *E. coli* ORN172(pIha) (A and C) or *E. coli* ORN172(pSK+) (B and D).

(Fig. 3). One of five human EPEC strains distantly related to *E. coli* O157:H7 has a *Bst*XI fragment detected by the *iha* probe that is slightly larger than the 4.7-kb *iha*-homologous *Bst*XI fragment in *E. coli* O157:H7 (Fig. 3). None of four *E. coli* O157:H− strains (*stx2*$^+$ pathogens closely related to *E. coli* O157:H7) and only one of seven *E. coli* O55:H7 strains (nontoxigenic EPEC closely related to *E. coli* O157:H7) tested contain *iha*-homologous DNA (Table 1 and Fig. 4). Only 3 of 20 commensal fecal *E. coli* strains have *iha*-homologous *Bst*XI fragments (Table 1).

The 35-kb insert of pSC(A-G6), which we have termed the tellurite resistance and adherence-conferring island (TAI), detects *Bst*XI fragments of identical size in 21 of 21 *E. coli* O157:H7 strains tested (Fig. 3 and 4). Six of seven *iha*$^+$ *eae*$^+$ STEC and RDEC-1 strains and one human EPEC strain tested also contain TAI homologues (defined as DNA in which most or all of the TAI-homologous *Bst*XI fragments are identical in size to TAI-homologous fragments in *E. coli* O157:H7 on Southern blots) (Fig. 3).

The TAI probe detects DNA in two *iha*$^+$ *eae*-negative STEC strains and one *iha*$^+$ *eae*$^+$ *E. coli* O55:H7 strain (Fig. 4), but the fragments detected differ in number and size from TAI-homologous *Bst*XI fragments in *E. coli* O157:H7. The TAI probe detects in some *iha*-negative *E. coli* strains, including *E. coli* HB101 (Fig. 3 and 4), *Bst*XI fragments that are fewer in number, fainter, and of different sizes than the TAI-homologous *Bst*XI fragments in *E. coli* O157:H7.

**OMP and TBP analysis.** *E. coli* ORN172(pIha) expresses a 78-kDa OMP, the deduced mass of the *iha*-encoded protein. *E. coli* ORN172(pSK+) does not express a similar protein. A prominent 67-kDa band is seen in *E. coli* O157:H7 OMPs (Fig. 5). α-Iha antibodies detect a 78-kDa OMP in *E. coli* ORN172(pIha) and a 67-kDa OMP in *E. coli* 0157:H7 strain 86-24 (Fig. 6 and 7) and each of eight additional *E. coli*


FIG. 2. Deduced amino acid sequence of Iha and homology to *V. cholerae* IrgA. The boxed amino acids represent a probable TonB box. +, conservative amino acid substitution. The arrows indicate the borders of amino acids deleted from Iha in *E. coli* O157:H7$^{nalR}$(Δ*iha*). The shaded amino acids were used to immunize rabbits to induce α-Iha antibodies. The dashes represent BLAST program-generated gaps between amino acids to maintain alignment of the proteins.

O157:H7 strains tested (data not shown), 56-kDa OMPs in *eae*-negative STEC O104:H21 and O113:H21 (Fig. 6B), and a faint 39-kDa OMP in *E. coli* O26:NM (Fig. 6A). α-Iha antibodies do not detect OMPs in other *iha*$^+$ *E. coli* tested or in *E. coli* ORN172(pSK+) (Fig. 6 and 7). α-Iha antibodies detect multiple peptides smaller than 78 kDa in some (Fig. 6A and 6B), but not all (Fig. 7), *E. coli* ORN172(pIha) OMP preparations. A 67-kDa band is not seen in Coomassie-stained OMPs from *E. coli*$^{nalR}$(Δ*iha*) (Fig. 5), but α-Iha antibodies detect a faint 67-kDa band in some (Fig. 7), but not all (Fig. 6A), of the OMPs prepared from this mutant.

We employed proteinase K digestion of whole bacteria to determine if Iha possesses proteinase-susceptible (i.e., externally directed) domains. Proteinase K digests the prominent 78- and 67-kDa and faint 67-kDa OMPs expressed by *E. coli* ORN172(pIha), *E. coli* O157:H7, and *E. coli* O157:H7$^{nalR}$(Δ*iha*), respectively, as seen on Coomassie blue-stained gels and protein immunoblots (Fig. 5 and 7). However, this treatment does not affect most other OMPs (Fig. 5) or periplasmic Mbp (data not shown). Hence, Iha has externally directed sites susceptible to proteinase digestion.

## DISCUSSION

Iha, an OMP with externally directed domains, is the first *E. coli* O157:H7 protein to be described that is sufficient to confer the adherence phenotype upon nonadherent laboratory *E. coli*. Iha is homologous to a variety of bacterial iron acquisition proteins in the database but not to other known adhesins. However, the homology between Iha and IrgA is significant because an *irgA*::Tn*phoA* mutant of *V. cholerae* colonizes infant mice less well than does its parent and is less virulent (16). Thus, of the many Iha-homologous proteins generated by the BLAST search, IrgA has a proposed function analogous to the adherence-conferring properties of Iha.

It is tempting to speculate that Iha homologues in organisms other than *E. coli* O157:H7 play a role in pathogenicity, especially in pathogens without *eae*. For example, ORF R4, an *iha* homologue found in *E. coli* CFT073 and other uropathogenic *E. coli* (17), might contribute to virulence by enhancing the adherence of these organisms. Also, *eae*-negative STEC, such as *E. coli* O104:H21 and *E. coli* O113:H21, which have caused epidemic (3, 31a) and sporadic (11) enteric human infections,

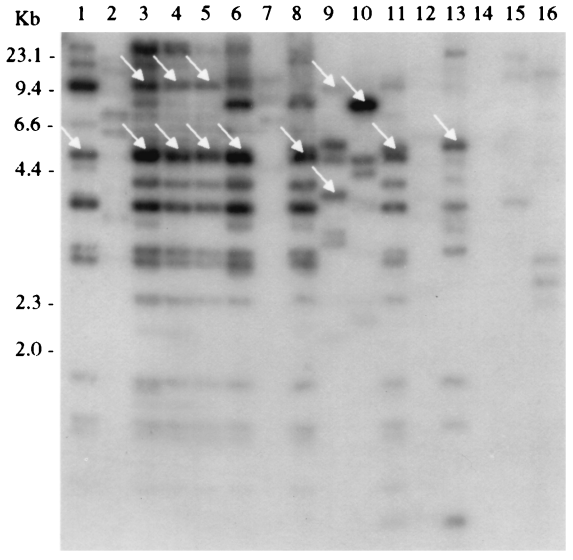


FIG. 3. Conservation of TAI in *E. coli* strains from diverse lineages. Hybridization of the 35-kb TAI probe to *Bst*XI-digested DNA from *E. coli* O157:H7 (lane 1), *E. coli* HB101 (lane 2), *E. coli* O26:NM (strain TB352A) (lane 3), *E. coli* O26:H2 (strain TB285A) (lane 4), *E. coli* O85:NM (strain TB334C) (lane 5), *E. coli* O103:H2 (UTI strain) (lane 6), *E. coli* O103:H6 (strain TB154A) (lane 7), *E. coli* O111:HN (strain TB226A) (lane 8), *E. coli* O104:H21 (lane 9), *E. coli* O113:H21 (strain CL15) (lane 10), *E. coli* O15:NM (strain RDEC-1) (lane 11), *E. coli* O111:NM (strain B171) (lane 12), *E. coli* O111:H− (strain 2430-78) (lane 13), *E. coli* O119:H6 (strain 659-79) (lane 14), *E. coli* O127:H6 (strain E2348-69) (lane 15), and *E. coli* O142:H6 (strain 581-71) (lane 16) is shown. The arrows indicate fragments detected when the membrane is probed with cloned *iha*.

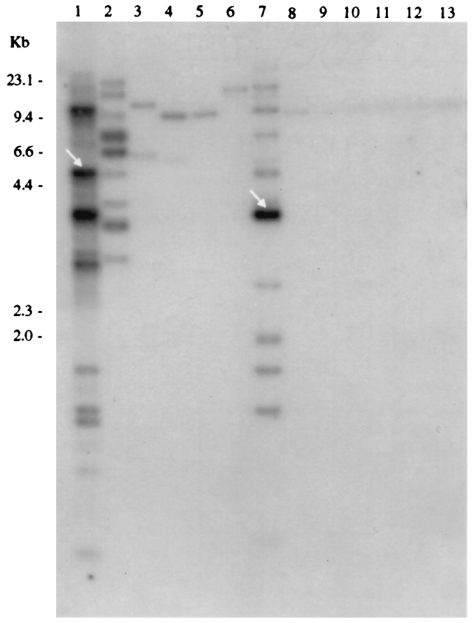


FIG. 4. Absence of TAI from *E. coli* strains closely related to *E. coli* O157:H7. Hybridization of the TAI probe to *Bst*XI-digested DNA from *E. coli* O157:H7 (lane 1); *E. coli* HB101 (lane 2); *E. coli* O55:H7 strains 5A, 5B, 5C, 5D, 5E, TB156A, and TB182A (lanes 3 to 9, respectively); and *E. coli* O157:H− strains 493-89, 5412-89, CB569, and 514-91 (lanes 10 to 13, respectively) is shown. The arrows indicate fragments detected when membrane is probed with cloned *iha*.

are *iha*$^+$ and have Iha antigen in their OMPs. Perhaps these *eae*-negative STEC utilize Iha or an Iha homologue for adherence and colonization purposes in lieu of intimin.

The possibility exists that *iha* does not encode an adhesin but instead encodes a protein that increases the expression of a cryptic adhesin in laboratory *E. coli*. Such a molecule might be analogous to Crl, a transcriptional activator of *csgA*, which encodes the curlin subunit enabling *E. coli* to bind fibronectin (4, 31). However, Crl is located predominantly in the cytoplasm, whereas Iha is a comparatively prominent *E. coli* OMP, as demonstrated by Coomassie blue staining and immunoblots. Furthermore, the susceptibility of Iha to proteinase K digestion following incubation of whole bacteria with this enzyme suggests that Iha possesses one or more externally exposed domains. These findings are all consistent with the role of Iha as an adhesin.

Technical difficulties precluded the performance of additional experiments that might have established more firmly the role of Iha as an adhesin. In particular, the demonstration that an antibody to an externally directed Iha epitope ablates or reduces epithelial cell adherence of *E. coli* O157:H7 or of *E. coli* ORN172(pIha) would help establish its role in adherence. However, the antibodies that we elicited by peptide immunization, after many unsuccessful attempts using other formulations of Iha as an immunogen, do not detect externally directed epitopes of this molecule, as they are expressed in *E. coli* O157:H7 or in laboratory *E. coli* (immunofluorescence data not shown). These α-Iha antibodies would not, therefore, be appropriate to use in adherence inhibition studies.

The smaller $M_r$ of the antigen detected by α-Iha antibodies in *E. coli* O157:H7 OMPs, compared to the $M_r$ of Iha deduced from the size of the adherence-conferring gene, suggests that full-length Iha might be cleaved in wild-type *E. coli* O157:H7. The array of immunoreactive proteins with $M_r$s smaller than the deduced size of Iha in some preparations of *E. coli* ORN172(pIha) OMPs also suggests that full-length Iha might be subject to either proteolytic cleavage or nonspecific degradation.

The lack of detectable Iha antigen in many non-O157:H7 *iha*$^+$ *E. coli* suggests the possibility of serotype- and lineage-specific expression of Iha. *E. coli* O157:H7 differs from non-O157:H7 STEC in its array of virulence genes (33), and perhaps also in the ability to express proteins encoded by shared alleles, such as *iha*. In fact, a precedent for serotype- and lineage-specific in vitro protein expression exists in the case of the EHEC hemolysin, which is encoded by a gene on pO157

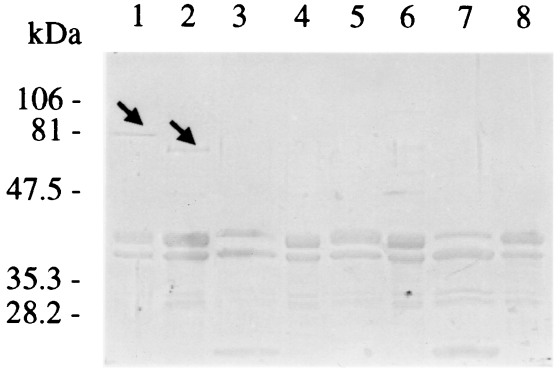


FIG. 5. Presence of Iha in OMPs of wild-type, mutant, and recombinant *E. coli*. Coomassie blue-stained OMPs of *E. coli* ORN172(pIha) (lanes 1 and 3), *E. coli* O157:H7 (lanes 2 and 4), *E. coli* ORN172(pSK+) (lanes 5 and 7), and *E. coli* O157:H7$^{nalR}$(Δ*iha*) (lanes 6 and 8) without (lanes 1, 2, 5, and 6) and with (lanes 3, 4, 7, and 8) proteinase K treatment. The arrows indicate probable Iha protein.

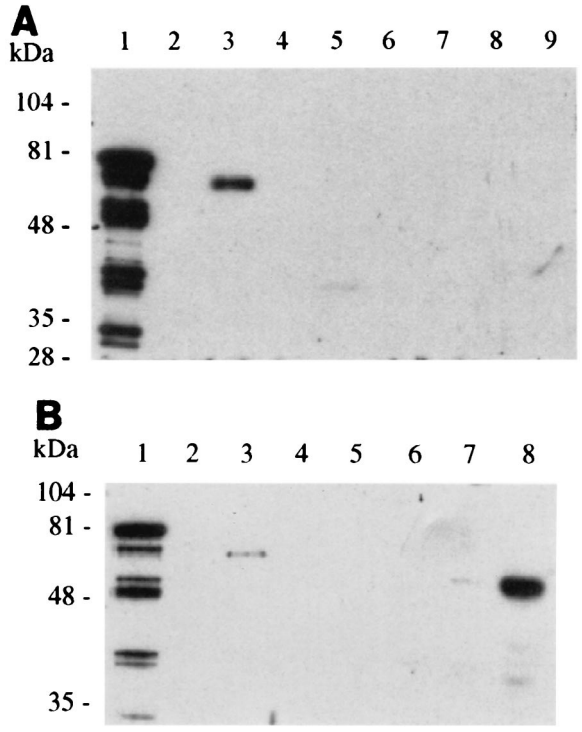


FIG. 6. Detection of immunoreactive Iha in *iha*$^+$ *E. coli* (A) OMPs from *E. coli* ORN172(pIha) (lane 1), *E. coli* ORN172(pSK+) (lane 2), *E. coli* O157:H7 (lane 3), *E. coli* O157:H7$^{nalR}$(Δ*iha*) (lane 4), *E. coli* O26:NM (strain TB352A) (lane 5), *E. coli* O26:H2 (strain TB285A) (lane 6), *E. coli* O85:NM (strain TB334C) (lane 7), *E. coli* O111:NM (strain TB226A) (lane 8), and *E. coli* O103:H2 (UTI strain) (lane 9) probed with α-Iha antibodies. (B) OMPs from *E. coli* ORN172(pIha) (lane 1), *E. coli* ORN172(pSK+) (lane 2), *E. coli* O157:H7 (lane 3), *E. coli* O55:H7 strain 5E (lane 4), *E. coli* O111:H− (strain 2430-78) (lane 5), *E. coli* O15:NM (strain RDEC-1) (lane 6), *E. coli* O104:H21 (lane 7), and *E. coli* O113:H21 (strain CL15) (lane 8) probed with α-Iha antibodies.

and on a similar plasmid in non-O157:H7 STEC (33) and which is variably expressed by pathogenic STEC. Alternatively, polymorphisms in expressed Iha homologues in non-O157:H7 STEC might interfere with the ability of α-Iha antibodies to detect these proteins.

Though Iha is sufficient to confer adherence, a virulence phenotype, upon nonadherent *E. coli*, we are cautious about designating Iha as a virulence factor. The role of Iha in the pathogenesis of *E. coli* O157:H7 might remain difficult to assign, because humans cannot be challenged with STEC or its derivatives. The possibility also exists that Iha facilitates the adherence of *E. coli* O157:H7 to epithelial cells in nonpathogenic milieus, such as animal gastrointestinal tracts. Indeed, *iha* confers upon laboratory *E. coli* the ability to adhere to MDBK cells, an epithelial line of bovine origin.

Our detection of a variably expressed OMP(s) that reacts with α-Iha antibodies raises the possibility that one or more yet to be characterized Iha homologues in *E. coli* O157:H7 also have adherence-conferring properties. Such a molecule might account for the residual and variable adherence of *E. coli* O157:H7 from which *iha* has been deleted. However, it should be noted that intimin, which clearly has a role in the intimate attachment of *E. coli* to epithelial cells, would probably be expressed by *E. coli* O157:H7$^{nalR}$(Δ*iha*) and could also have mediated this residual adherence.

The phylogenetic aspects of the acquisition of *iha* and TAI are noteworthy. The presence and conserved structure of TAI in multiple *E. coli* strains distantly related to *E. coli* O157:H7 suggest that this island transfers between organisms on a mobile element. Our data also suggest that *E. coli* O157:H7 acquired TAI relatively recently, i.e., after it acquired the O157 *rfb* cluster and the *stx2*-encoding bacteriophage in its evolution from the progenitor it shares with *E. coli* O55:H7. TAI acquisition therefore represents an additional differentiating event in the evolution of *E. coli* O157:H7 from *E. coli* O157:H− (39).

Our data provide a genetic explanation for the respective tellurite resistances and susceptibilities of *E. coli* O157:H7 and *E. coli* O157:H− (22, 43). Tellurite resistance loci and the Iha-homologous colicin I receptor are linked on plasmid R478 in *S. marcescens*. This plasmid confers adherence on *E. coli* J62 (29), possibly via this Iha homologue. However, in the *E. coli* CFT073 PAI, tellurite resistance loci are not found 3′ of an *iha* homologue (20), so the linkage between genes encoding tellurite resistance and *iha* homologues is not always conserved.

In summary, our work introduces Iha as a novel adherence-conferring molecule. In its evolution from the progenitor it shares with *E. coli* O55:H7, and in its differentiation from *E. coli* O157:H−, *E. coli* O157:H7 acquired TAI, and possibly other chromosomal islands, in addition to toxin-encoding bacteriophages and *rfb* loci. The role of Iha in animal and human colonization by STEC, the structure and function of the Iha homologues in a variety of bacteria, the mechanisms underlying Iha expression, and the mode of transfer of TAI between bacteria warrant further elucidation.

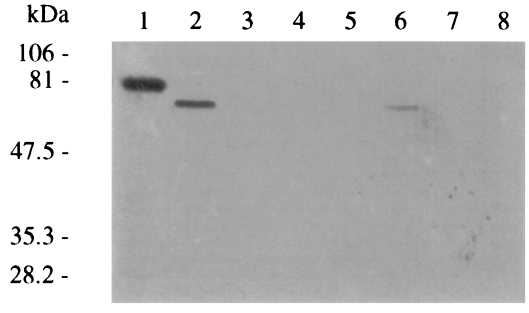


FIG. 7. Effect of proteinase K on immunoreactive Iha. OMPs of *E. coli* ORN172(pIha) (lanes 1 and 3), *E. coli* O157:H7 (lanes 2 and 4), *E. coli* ORN172(pSK+) (lanes 5 and 7), and *E. coli* O157:H7$^{nalR}$(Δ*iha*) (lanes 6 and 8) without (lanes 1, 2, 5, and 6) and with (lanes 3, 4, 7, and 8) proteinase K treatment, probed with α-Iha antibodies.


## ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (AI-38419), U.S. Department of Agriculture (94-03953), and National Cattlemen's Beef Association grants.

We thank Steve L. Moseley for generous advice and encouragement; Stephen B. Calderwood, Michael S. Donnenberg, James B. Kaper, Beth Traxler, Thomas S. Whittam, and Shing-Erh Yen for helpful suggestions; and Christine A. Merrikin for secretarial assistance.


## REFERENCES


1. **Achtman, M., A. Mercer, B. Kusecek, A. Pohl, M. Heuzenroeder, W. Aaronson, A. Sutton, and R. P. Silver.** 1983. Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infect. Immun. **39:**315–335.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Anonymous.** 1994. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21—Helena, Montana, 1994. Morbid. Mortal. Weekly. Rep. **44:**501–503.
4. **Arnqvist, A., A. Olsen, J. Pfeifer, D. G. Russell, and S. Abrmark.** 1992. The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101. Mol. Microbiol. **6:**2443–2452.
5. **Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr,**

**C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, et al.** 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. JAMA **272:**1349–1353.
6. **Bilge, S. S., J. C. Vary, Jr., S. F. Dowell, and P. I. Tarr.** 1996. Role of the *Escherichia coli* O157:H7 O side chain in adherence and analysis of an *rfb* locus. Infect. Immun. **64:**4795–4801.
7. **Bokete, T. N., T. S. Whittam, R. A. Wilson, C. R. Clausen, C. M. O'Callahan, S. L. Moseley, T. R. Fritsche, and P. I. Tarr.** 1997. Genetic and phenotypic analysis of *Escherichia coli* with enteropathogenic characteristics isolated from Seattle children. J. Infect. Dis. **175:**1382–1389.
8. **Cantey, J. R., and R. K. Blake.** 1977. Diarrhea due to *Escherichia coli* in the rabbit: a novel mechanism. J. Infect. Dis. **135:**454–462.
9. **Dauenhauer, S. A., R. A. Hull, and R. P. Williams.** 1984. Cloning and expression in *Escherichia coli* of *Serratia marcescens* genes encoding prodigiosin biosynthesis. J. Bacteriol. **158:**1128–1132.
10. **Donnenberg, M. S., and J. B. Kaper.** 1991. Construction of an *eae* deletion mutant of enteropathogenic *Escherichia coli* by using a positive-selection suicide vector. Infect. Immun. **59:**4310–4317.
11. **Dytoc, M. T., A. Ismaili, D. J. Philpott, R. Soni, J. L. Brunton, and P. M. Sherman.** 1994. Distinct binding properties of *eaeA*-negative verocytotoxin-producing *Escherichia coli* of serotype O113:H21. Infect. Immun. **62:**3494–3505.
12. **Elliott, S. J., J. Yu, and J. B. Kaper.** 1999. The cloned locus of enterocyte effacement from enterohemorrhagic *Escherichia coli* O157:H7 is unable to confer the attaching and effacing phenotype upon *E. coli* K-12. Infect. Immun. **67:**4260–4263.
13. **Gish, W., and D. J. States.** 1993. Identification of protein coding regions by database similarity search. Nat. Genet. **3:**266–272.
14. **Glantz, S. A.** 1997. Primer of biostatistics, 4th ed. McGraw-Hill Health Professions, New York, N.Y.
15. **Goldberg, M. B., S. A. Boyko, J. R. Butterton, J. A. Stoebner, S. M. Payne, and S. B. Calderwood.** 1992. Characterization of a *Vibrio cholerae* virulence factor homologous to the family of TonB-dependent proteins. Mol. Microbiol. **6:**2407–2418.
16. **Goldberg, M. B., V. J. DiRita, and S. B. Calderwood.** 1990. Identification of an iron-regulated virulence determinant in *Vibrio cholerae*, using Tn*phoA* mutagenesis. Infect. Immun. **58:**55–60.
17. **Guyer, D. M., J. S. Kao, and H. L. Mobley.** 1998. Genomic analysis of a pathogenicity island in uropathogenic *Escherichia coli* CFT073: distribution of homologous sequences among isolates from patients with pyelonephritis, cystitis, and catheter-associated bacteriuria and from fecal samples. Infect. Immun. **66:**4411–4417.
18. **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.
19. **Jobling, M. G., and D. A. Ritchie.** 1988. The nucleotide sequence of a plasmid determinant for resistance to tellurium anions. Gene **66:**245–258.
20. **Kao, J. S., D. M. Stucker, J. W. Warren, and H. L. Mobley.** 1997. Pathogenicity island sequences of pyelonephritogenic *Escherichia coli* CFT073 are associated with virulent uropathogenic strains. Infect. Immun. **65:**2812–2820.
21. **Kaper, J. B., S. Elliott, V. Sperandio, N. T. Perna, G. F. Mayhew, and F. R. Blattner.** 1998. Attaching-and-effacing intestinal histopathology and the locus of enterocyte effacement, p. 163–182. *In* J. B. Kaper and A. D. O'Brien (ed.), *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli*. ASM Press, Washington, D.C.
22. **Karch, H., C. Janetzki-Mittmann, S. Aleksic, and M. Datz.** 1996. Isolation of enterohemorrhagic *Escherichia coli* O157 strains from patients with hemolytic-uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. J. Clin. Microbiol. **34:**516–519.
23. **Levine, M. M., J. P. Nataro, H. Karch, M. M. Baldini, J. B. Kaper, R. E. Black, M. L. Clements, and A. D. O'Brien.** 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. J. Infect. Dis. **152:**550–559.
24. **Levine, M. M., J. G. Xu, J. B. Kaper, H. Lior, V. Prado, B. Tall, J. Nataro, H. Karch, and K. Wachsmuth.** 1987. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. J. Infect. Dis. **156:**175–182.
25. **Louie, M., J. C. de Azavedo, M. Y. Handelsman, C. G. Clark, B. Ally, M. Dytoc, P. Sherman, and J. Brunton.** 1993. Expression and characterization of the *eaeA* gene product of *Escherichia coli* serotype O157:H7. Infect. Immun. **61:**4085–4092.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **McDaniel, T. K., and J. B. Kaper.** 1997. A cloned pathogenicity island from enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K-12. Mol. Microbiol. **23:**399–407.
28. **McKee, M. L., and A. D. O'Brien.** 1996. Truncated enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 intimin (EaeA) fusion proteins promote adherence of EHEC strains to HEp-2 cells. Infect. Immun. **64:**2225–2233.
29. **Mignatti, P., L. Pagani, M. Perduca, and E. Romero.** 1985. R factor-mediated adhesiveness to mammalian cells in *E. coli* K12. Microbiologica **8:**101–111.
30. **Newland, J. W., and R. J. Neill.** 1988. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. J. Clin. Microbiol. **26:**1292–1297.
31. **Olsen, A., A. Jonsson, and S. Normark.** 1989. Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. Nature **20:**652–655.
31a. **Paton, A. W., M. C. Woodrow, R. M. Doyle, J. A. Lanser, and J. C. Paton.** 1999. Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic-uremic syndrome. J. Clin. Microbiol. **37:**3357–3361.
32. **Riley, L. W., L. N. Junio, L. B. Libaek, and G. K. Schoolnik.** 1987. Plasmid-encoded expression of lipopolysaccharide O-antigenic polysaccharide in enteropathogenic *Escherichia coli*. Infect. Immun. **55:**2052–2056.
33. **Schmidt, H., C. Geitz, P. I. Tarr, M. Frosch, and H. Karch.** 1999. Non-O157:H7 pathogenic Shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. J. Infect. Dis. **179:**115.
34. **Simon, R., U. Priefer, and A. Puhler.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. BioTechniques **1:**784–791.
35. **Tarr, P. I., and S. S. Bilge.** 1998. Intimin-independent adherence mechanisms of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains, p. 157–162. *In* J. B. Kaper, and A. D. O'Brien (ed.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli*. ASM Press, Washington, D.C.
36. **Tarr, P. I., L. S. Fouser, A. E. Stapleton, R. A. Wilson, H. H. Kim, J. C. Vary, Jr., and C. R. Clausen.** 1996. Hemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga-toxin-producing *Escherichia coli* O103:H2. N. Engl. J. Med. **335:**635–638.
37. **Tarr, P. I., M. A. Neill, C. R. Clausen, J. W. Newland, R. J. Neill, and S. L. Moseley.** 1989. Genotypic variation in pathogenic *Escherichia coli* O157:H7 isolated from patients in Washington, 1984–1987. J. Infect. Dis. **159:**344–347.
38. **Wahl, G. M., K. A. Lewis, J. C. Ruiz, B. Rothenberg, J. Zhao, and G. A. Evans.** 1987. Cosmid vectors for rapid genomic walking, restriction mapping and gene transfer. Proc. Natl. Acad. Sci. USA **84:**2160–2164.
39. **Whittam, T. S.** 1995. Genetic population structure and pathogenicity in enteric bacteria, p. 217–245. *In* S. Baumberg, J. P. W. Young, S. R. Saunders, and E. M. H. Wellington (ed.), Population genetics of bacteria. Cambridge University Press, Cambridge, United Kingdom.
40. **Whittam, T. S., and E. A. McGraw.** 1996. Clonal analysis of EPEC serogroups. Rev. Microbiol. **27:**7–16.
41. **Whittam, T. W., M. L. Wolfe, I. K. Wachsmuth, F. Orskov, I. Orskov, and R. A. Wilson.** 1993. Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. Infect. Immun. **61:**1619–1629.
42. **Woodall, L. D., P. W. Russell, S. L. Harris, and P. E. Orndorff.** 1993. Rapid, synchronous, and stable induction of type 1 piliation in *Escherichia coli* by using a chromosomal lacUV5 promoter. J. Bacteriol. **175:**2770–2778.
43. **Zadik, P. M., P. A. Chapman, and C. A. Siddons.** 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J. Med. Microbiol. **39:**155–158.

*Editor:* P. E. Orndorff