Anaerobic Conditions Promote Expression of Sfp Fimbriae and Adherence of Sorbitol-Fermenting Enterohemorrhagic Escherichia coli O157:NM to Human Intestinal Epithelial Cells

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The sfp gene cluster, unique to sorbitol-fermenting (SF) enterohemorrhagic Escherichia coli (EHEC) O157:NM strains, encodes fimbriae that mediate mannose-resistant hemagglutination in laboratory E. coli strains but are not expressed in wild-type SF EHEC O157:NM strains under standard laboratory conditions. We investigated whether Sfp fimbriae are expressed under conditions that mimic the intestinal environment and whether they contribute to the adherence of SF EHEC O157:NM strains to human intestinal epithelial cells. The transcription of sfpA (encoding the major fimbrial subunit) was upregulated in all strains investigated, and all expressed SfpA and possessed fimbriae that reacted with an anti-SfpA antibody when the strains were grown on solid media under anaerobic conditions. Sfp expression was absent under aerobic conditions and in liquid media. Sfp upregulation under anaerobic conditions was significantly higher on blood agar and a medium simulating the colonic environment than on a medium simulating the ileal environment (P < 0.05). The induction of Sfp fimbriae in SF E. coli O157:NM strains correlates with increased adherence to Caco-2 and HCT-8 cells. Our data indicate that the expression of Sfp fimbriae in SF E. coli O157:NM strains is induced under conditions resembling those of the natural site of infection and that Sfp fimbriae may contribute to the adherence of the organisms to human intestinal epithelium.

Enterohemorrhagic Escherichia coli (EHEC) strains of serogroup O157, which cause hemorrhagic colitis and hemolytic-uremic syndrome (HUS), differ in their capacity to ferment the sugar alcohol sorbitol. Strains that do not ferment sorbitol are mostly serotype O157:H7 (51), whereas sorbitol-fermenting (SF) E. coli O157 strains belong to the class of nonmotile strains (O157:NM) (29). Whereas EHEC O157:H7 strains are mostly serotype O157:H7 (51), whereas sorbitol-fermenting (SF) EHEC O157:NM strains (O157:NM) (29). Whereas EHEC O157:H7 strains are mostly serotype O157:H7 (51), whereas sorbitol-fermenting (SF) EHEC O157:NM strains (O157:NM) (29). Whereas EHEC O157:H7 strains are mostly serotype O157:H7 (51).

The induction of Sfp fimbriae in SF E. coli O157:NM strains correlates with increased adherence to Caco-2 and HCT-8 cells. Our data indicate that the expression of Sfp fimbriae in SF E. coli O157:NM strains is induced under conditions resembling those of the natural site of infection and that Sfp fimbriae may contribute to the adherence of the organisms to human intestinal epithelium.

MATERIALS AND METHODS

Bacterial strains. Recombinant E. coli strain HB101/pSFO157-E11 harbors a 10.9-kb EcoRI fragment of plasmid pSFO157 from SF EHEC O157:NM strain 3072/96 containing the sfp gene cluster which has been cloned into the pBluescript II KS(+) vector (Stratagene, La Jolla, CA) (12). Wild-type SF E. coli O157:NM strains 493/89, 3072/96, and El03/71 were isolated from stool specimens

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of patients with HUS (9, 12, 29). The first two strains were 
str+, positive whereas the third lacks str; all strains contain eae, encoding the adhesin intimin, and a complete sfp cluster. E. coli HB101 harboring the pBluescript II KS(+) vector without any insert was used as a control.

Culture conditions. To investigate the influence of a change in the environmental conditions on the expression of Sfp fimbrae, strains were passaged four times (24 h, 37°C) on Luria-Bertani (LB) agar, Columbia blood agar (Heipha, Heidelberg, Germany), simulated-ileal-environment medium (SIEM), and simulated-colonic-environment medium (SCM) under standard aerobic and anaerobic (Anaerocult A; Merck, Darmstadt, Germany) conditions. SIEM and SCM were prepared as described elsewhere (6) with minor modifications. SIEM (pH 7.0) contained 5.7 g of Bacto tryptone (Difco, Hamburg, Germany)/liter, 2.4 g of try-glucose/liter, 6.14 g of NaCl/liter, 0.68 g of KH₂PO₄/liter, 0.3 g of Na₂HPO₄/liter, 1.01 g of NaHCO₃/liter, 5.6 g of bile salts/liter, 0.2 g of lysozyme/liter, 1,000 U of α-amylase/liter, 110 U of trypsin/liter, and 380 U of chymotrypsin/liter. SCM (pH 7.0) contained Bacto tryptone at 6.25 g/liter, r-glucose at 2.6 g/liter, NaCl at 0.88 g/liter, KH₂PO₄ at 0.43 g/liter, NaHCO₃ at 1.7 g/liter, KHCO₃ at 2.7 g/liter, and bile salts at 4.0 g/liter. Solid media were prepared by adding 1.5% (wt/vol) Bacto agar (Difco). In some experiments, bacteria were also cultured in LB broth or CDMT medium [13 mM KH₂PO₄, 6 mM KH₂PO₄, 8 mM (NH₄)₂SO₄, 2 mM sodium citrate, 0.4 mM MgSO₄, 0.2% Casamino Acids, 0.2% glucose, 5 μM CaCl₂, 0.01% trypsin (pH 7.4)] (12). Amincillin (100 μg/ml) was added to media used to culture HB101/pSFO157-E11.

Isolation of Sfp fimbrae. Sfp fimbrae were isolated from the HB101/pSFO157-E11 and wild-type SF. coli O157/NM strains as described elsewhere (12). Briefly, after four passages on blood agar, bacteria were enriched for 24 h in CDMT medium under aerobic and anaerobic (Anaerocult A) conditions, harvested by centrifugation, and resuspended in a 1/10 volume of a solution containing 75 mM NaCl and 0.5 mM Tris-HCl (pH 7.4). After heating (60°C, 90 min), bacteria were pelleted by centrifugation, and the thermoequilibrated proteins in the supernatant were precipitated with trichloroacetic acid and dissolved in Laemmli sample buffer (34).

SDS-PAGE and immunoblotting. The Sfp preparations were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (34) and 5% SDS gel slab gel apparatus (Laemmli, Munich, Germany). Gels were stained with Coomassie blue, or unstained proteins were transferred to a nitrocellulose membrane (Roht, Karlsruhe, Germany) for immunoblot analysis. Immunoblotting used a rabbit antibody against SfpA (12) diluted 1:2,000 and an alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G antibody (Dianova, Hamburg, Germany) diluted 1:2,000.

Quantitative RT-PCR assay. Total RNA was isolated with the RNeasy minikit (Qiagen, Hilden, Germany). One-step quantitative reverse transcription PCR (RT-PCR), performed with the LightCycler system (Roche, Mannheim, Germany) and the Quantitect SYBR green RT-PCR kit (Qiagen), was used to determine the relative expression levels of sfpA mRNA. The PCRs were performed in 10 μl containing 1 μl of total RNA (20 ng), 5 μl of 2× Quantitect SYBR green RT-PCR master mix, 0.1 μl of Quantitect RT mix, 4 mM MgCl₂, and 0.5 μM each of the forward primer (5'-TTTTGGATTATG-3') and the reverse primer (5'-TTCTGCGGT-3'). The PCR products were amplified with primers GapA Forward and GapA Reverse. One-step RT-PCR included a reverse transcription step at 50°C for 20 min and preliminary denaturation at 95°C for 15 min. The PCR products were amplified with the following conditions: 40 cycles of denaturation (94°C, 10 s), annealing (52°C, 10 s), and extension (72°C, 20 s). For each mRNA assay, relative quantification was achieved using an external standard (purified gapA) and the respective sfpA PCR products. Data were analyzed using the fit point method with LightCycler (version 3.5) software. sfpA mRNAs were normalized to gapA mRNA. Control reactions were performed without reverse transcriptase to confirm that the target detection was RNA.

Hemagglutination assay. Medium-sized colonies from cultures grown aerobiically and anaerobically on solid medium (LB agar, Columbia blood agar, SIEM, or SCM) were mixed with 15 μl of cultures grown in liquid medium (LB broth, CDMT, SIEM, or SCM) and were washed and fresh medium containing 0.5% D-mannose was added, cultures were infected with bacteria (1 × 10⁶ CFU) that had been passaged four times (24 h, 37°C) under aerobic or anaerobic (Anaerocult A) conditions either in liquid medium (CDMT, SIEM, or SCM) or on solid medium (Columbia blood agar, SIEM, or SCM). The cells were incubated with bacteria (37°C, 5% CO₂ for 2 h (Caco-2) or 4.5 h (HCT-8)); these incubation periods corresponded to the time intervals, determined in preliminary experiments, over which the vector control strain showed no adherence. The cells were then washed thoroughly with phosphate-buffered saline (PBS) (Cambrex Bioscience, Verviers, Belgium), fixed with 70% ethanol, and stained with 10% Giemsa stain (Merck, Darmstadt, Germany). The cover slides were mounted using Glycergel medium (DakoCytomation, Hamburg, Germany), and bacterial adherence was examined using light microscopy (Axio Imager A1; Zeiss, Jena, Germany) and photographed (AxioCam MRm camera; Zeiss).

Electron microscopy. A single bacterial colony, passaged four times on blood agar under aerobic and anaerobic conditions, was applied to Formvar-coated copper grids. Bacteria were allowed to sediment for 5 min and were then negatively contrasted with 1% phosphotungstic acid. For immunogold staining, bacteria were incubated for 2 h with an anti-SfpA antibody diluted 1:20 in Dulbecco’s PBS (D-PBS) containing 10% bovine serum albumin (Sigma). After three washes with D-PBS, immunogold labeling was performed for 45 min using a goat anti-rabbit antibody absorbed with 12-nm diameter gold particles (Dianova, Hamburg, Germany) diluted 1:50 in D-PBS. After washes with D-PBS and then with distilled water, the bacteria were contrasted with 1% phosphotungstic acid in distilled water for 45 to 50 s. The samples were analyzed using a Tecnai G2 Spirit Twin electron microscopy (FEI Company, Hillsboro, OR) (magnification, ×21,000 to ×240,000).

Results Effects of culture conditions on MRHA caused by SF. coli O157/NM. When grown on LB agar, blood agar, or a solid medium simulating the ileal (SIEM) or colonic (SCM) environment under aerobic conditions, none of the wild-type SF. coli O157/NM strains 493/89, 3072/96, and E03/71 caused MRHA (Table 1). However, the MRHA phenotype could be induced in all strains by passaging on the respective media under anaerobic conditions (Table 1). The ability to cause MRHA became apparent after the second passage and reached a maximum in all strains and with all media after four passages. The intensity of the MRHA (Table 1) was observed in all strains and media with the medium simulating the ileal (SIEM). All strains were infected with bacteria (1 × 10⁶ CFU) that had been passaged four times (24 h, 37°C) under aerobic or anaerobic (Anaerocult A) conditions either in liquid medium (CDMT, SIEM, or SCM) or on solid medium (Columbia blood agar, SIEM, or SCM). The cells were incubated with bacteria (37°C, 5% CO₂ for 2 h (Caco-2) or 4.5 h (HCT-8)); these incubation periods corresponded to the time intervals, determined in preliminary experiments, over which the vector control strain showed no adherence. The cells were then washed thoroughly with phosphate-buffered saline (PBS) (Cambrex Bioscience, Verviers, Belgium), fixed with 70% ethanol, and stained with 10% Giemsa stain (Merck, Darmstadt, Germany). The cover slides were mounted using Glycergel medium (DakoCytomation, Hamburg, Germany), and bacterial adherence was examined using light microscopy (Axio Imager A1; Zeiss, Jena, Germany) and photographed (AxioCam MRm camera; Zeiss).
observed for wild-type SF E. coli O157:NM strains grown in liquid media, including LB broth and CDM, under either aerobic or anaerobic conditions (data not shown); repeated passages (as many as six) had no effect on this result. The MRHA abilities of liquid cultures grown in SIEM or SCEM could not be determined, because these cultures rapidly hemolyzed erythrocytes. The sfp-positive strain HB101/pSFO157-E11 caused strong MRHA after a single passage regardless of the medium and culture conditions used, but MRHA was not observed with the vector control strain (Table 1).

**Quantitative RT-PCR analysis of sfpA mRNA in SF E. coli O157:NM strains grown under aerobic and anaerobic conditions.** Strains 493/89, 3072/96, and E03/71, passaged four times using blood agar, SIEM, and SCEM under anaerobic conditions, showed increases in sfpA transcription of 1.9- to 3.4-fold, 1.4- to 3.1-fold, and 3.0-fold, respectively, over that observed under aerobic conditions (Fig. 1). These differences were significant (P < 0.05) for cultures on blood agar and SCEM (Fig. 1). The upregulation of sfpA transcription in strains 493/89 and 3072/96, which was examined using all three media, was most pronounced in cultures grown on blood agar, intermediate in cultures on SCEM, and lowest in cultures on SIEM (Fig. 1). Whereas the upregulation of sfpA transcription on blood agar and SCEM did not differ significantly, the transcription of sfpA with these two media was greater than that with SIEM (P < 0.05) (Fig. 1). sfpA transcription in HB101/pSFO157-E11 was constitutive under the conditions (media and culture atmosphere) used and was 57-fold to 187-fold elevated (under aerobic conditions (Fig. 1)). These differences were 1.4- to 3.1-fold, and 3.0-fold, respectively, over that observed with the vector control strain (Table 1).

**TABLE 1. Influence of anaerobic conditions on the expression of Sfp fimbriae in wild-type SF E. coli O157:NM strains and the sfp-positive HB101/pSFO157-E11 clone in culture on solid media.**

| Strain and culture conditions<sup>a</sup> | MRHA<sup>b</sup> on: | SfpA immunoblot result<sup>c</sup> | Presence of Sfp fimbriae<sup>d</sup> |
|----------------------------------------|------------------|-------------------------------|-------------------------------|
|                                        | LB agar | BA | SIEM | SCEM |                                        |                                        |
| 493/89                                 |         |   |      |      | -               |                                                     |
| Aerobic                                | -       | -  | -    | -    | -               |                                                     |
| Anaerobic                              | +       | ++ | +    | +    | +               |                                                     |
| 3072/96                                |         |   |      |      | -               |                                                     |
| Aerobic                                | -       | -  | -    | -    | -               |                                                     |
| Anaerobic                              | +       | ++ | +    | +    | +               |                                                     |
| E03/71                                 |         |   |      |      | -               |                                                     |
| Aerobic                                | -       | -  | -    | -    | -               |                                                     |
| Anaerobic                              | +       | ++ | NP   | NP   | +               |                                                     |
| HB101/pSFO157-E11 sfp<sup>+</sup> clone|         |   |      |      | -               |                                                     |
| Aerobic                                | -       | -  | -    | -    | -               |                                                     |
| Anaerobic                              | +       | ++ | NP   | NP   | +               |                                                     |
| Vector control [HB101/pBluescript II   |         |   |      |      | -               |                                                     |
| KS(++)                                 |         |   |      |      | -               |                                                     |
| Aerobic                                | -       | -  | -    | -    | -               |                                                     |
| Anaerobic                              | -       | -  | -    | -    | -               |                                                     |

<sup>a</sup> Anaerocult A (Merck) was used for anaerobic culture.

<sup>b</sup> Observed after four passages. BA, Columbia blood agar. The intensities of the MRHA reactions were classified as follows: ++++, immediate, complete, very strong HA (large aggregates involving all erythrocytes); ++, immediate, complete, strong HA (smaller aggregates involving all erythrocytes); +, immediate, incomplete HA (smaller aggregates involving most but not all erythrocytes); -, no HA observed; NP, not performed.

<sup>c</sup> Expressed as the binding of an anti-SfpA antibody to an 18-kDa band of Sfp fimbrial preparations from cultures passed four times on blood agar. +++, strong binding; +, weaker binding; -, no binding.

<sup>d</sup> As determined by electron microscopy of bacteria passed four times on blood agar using negative staining and immunogold staining. +++, numerous fimbriae detected on the surfaces of bacteria (examples in Fig. 2A and B, panels 1); +, less frequent fimbriae detected on the surfaces of bacteria (examples in Fig. 2A and B, panels 3); -, no fimbriae detected (examples in Fig. 2A and B, panels 2 and 4).

**FIG. 1. Influence of anaerobiosis on sfpA transcription in SF E. coli O157:NM strains.** Strains were passaged four times on the media indicated under aerobic or anaerobic (Anaerocult A) conditions, and sfpA mRNA was quantified using RT-PCR. *, sfpA transcription under anaerobic conditions was significantly higher than that under aerobic conditions on blood agar for strains 493/89 (P = 0.015), 3072/96 (P = 0.038), and E03/71 (P = 0.038). **, sfpA transcription under anaerobic conditions was significantly higher than that under aerobic conditions on SCEM for strains 493/89 (P = 0.020) and 3072/96 (P = 0.038). The upregulation of sfpA mRNA was significantly higher on blood agar and SCEM than on SIEM for strain 493/89 (P = 0.020 and 0.038, respectively) and significantly higher on blood agar than on SIEM for strain 3072/96 (P = 0.038). All data are means from three independent experiments. Error bars, standard deviations.
higher than that in the wild-type SF *E. coli* O157:NM strains (data not shown). No *sfpA* transcripts were elicited in the vector control strain (Fig. 1).

**Immunoblot detection of SfpA in SF *E. coli* O157:NM strains grown under aerobic and anaerobic conditions.** The SfpA protein was not detected in Sfp preparations of any of the wild-type strains grown aerobically (Table 1). In contrast, in all strains grown anaerobically, an 18-kDa band in the Sfp fimbrial preparations, corresponding in size to SfpA (12), was detected by the SfpA antibody (Table 1). In accordance with the lower *sfpA* transcription in the wild-type *E. coli* O157:NM strains than in HB101/pSFO157-E11 (see above), the signals elicited from the wild-type strains were substantially weaker than that from the clone (Table 1). For the HB101/pSFO157-E11 clone, in which the transcription of *sfpA* was constitutive, the intensities of the SfpA immunoblot signals for cultures grown aerobically and anaerobically were similar (Table 1).

**MALDI-TOF MS analysis of SfpA protein.** The identification of the 18-kDa SDS-PAGE band that reacted with the anti-SfpA antibody in HB101/pSFO157-E11 and in wild-type *SF E. coli* O157:NM strains grown anaerobically as the SfpA protein was verified by analyzing the fragments of the enzymatic cleavage of this band using MALDI-TOF MS. Combined data from tryptic digestion and simultaneous digestion with trypsin and Glu-C demonstrated that the amino acid sequence of the band corresponded (with 81% sequence coverage) to that predicted for the SfpA protein from HB101/pSFO157-E11 (UniProt/TrEMBL entry Q7B2V9).

**Electron microscopic evidence of Sfp fimbriae in wild-type SF *E. coli* O157:NM strains.** After negative staining, numerous fimbriae with diameters of ~3 nm were observed for HB101/pSFO157-E11 in both anaerobic (Fig. 2A, panel 1) and aerobic (electron microscopic data not shown) cultures (Table 1). Very fine fimbriae, with diameters similar to those in HB101/pSFO157-E11 but much less numerous, were also present in strain 493/89 when the strain was cultured anaerobically (Fig. 2A, panel 3). However, no fimbriae could be demonstrated in strain 493/89 grown aerobically (Fig. 2A, panel 4). Fimbriae were also absent from the vector control strain when grown anaerobically (Fig. 2A, panel 2) and aerobically (electron microscopic data not shown) (Table 1).

Confirmation that the structures expressed by HB101/pSFO157-E11 and strain 493/89 were Sfp fimbriae was obtained by immunogold staining with an anti-SfpA antibody (Fig. 2B, panels 1 and 3, respectively). The difference in the intensity of the gold labeling between the clone (Fig. 2B, panel 1) and strain 493/89 (Fig. 2B, panel 3) corresponded to the differences in the number of fimbriae after negative staining (Fig. 2A, panels 1 and 3, respectively). Strain 493/89 cultured aerobically showed no gold staining (Fig. 2B, panel 4), in accordance with the absence of fimbriae in the negatively stained strain grown under the same conditions (Fig. 2A, panel 4). Results similar to those obtained with strain 493/89 were obtained with SF *E. coli* O157:NM strain 3072/96 (Table 1), but the expression of Sfp fimbriae under anaerobic conditions was less than that in strain 493/89. Immunogold staining was absent from the vector control strain under both anaerobic (Fig. 2B, panel 2) and aerobic (data not shown) conditions.

**Correlation between expression of Sfp fimbriae and adherence to intestinal epithelium.** To investigate if Sfp fimbriae contribute to the adherence of *SF E. coli* O157:NM to human intestinal epithelial cells, strains 493/89, 3072/96, and E03/71, grown under conditions that did or did not induce expression of Sfp fimbriae, were tested for their capacities to adhere to Caco-2 and HCT-8 cells. None of the strains grown aerobically on solid medium adhered (Table 2), whereas all wild-type strains in which the expression of Sfp fimbriae had been induced by four passages under anaerobic conditions (as demonstrated by RT-PCR, immunoblotting, and electron microscopy) adhered to HCT-8 cells (Table 2; Fig. 3A, panel 4). As predicted by the quantitative RT-PCR analysis of *sfpA* mRNA (Fig. 1), cultures from SIEM adhered less intensively to HCT-8 cells than those from blood agar and SCEM, and they did not adhere at all to Caco-2 cells (Table 2). The intensity of the adherence of the wild-type strains was substantially less (Fig. 3A and B, panels 4) than that of HB101/pSFO157-E11 (Fig.
TABLE 2. Influence of culture conditions on the adherence of wild-type SF. coli O157:NM strains and the sfp-positive HB101/pSFO157-E11 clone to human intestinal epithelial cell lines

| Strain and culture conditions<sup>a</sup> | Adherence of bacteria<sup>b</sup> grown on the indicated medium to the following cell line (exposure time<sup>c</sup>): |  |
|----------------------------------------|---------------------------------------------------|---|
|                                        | BA SIEM SCEM BA SIEM SCEM                         |  |
| 493/89                                 | Aerobic: - - - - - - |  |
|                                        | Anaerobic: + + + + + |  |
| 3072/96                                | Aerobic: - - - - - - |  |
|                                        | Anaerobic: + + + + + |  |
| E03/71                                 | Aerobic: - - - - - - |  |
|                                        | Anaerobic: + + + + + |  |
| HB101/pSFO157-E11 sfp<sup>+</sup> clone| Aerobic: ++++++ +++ |  |
|                                        | Anaerobic: ++++++ +++ |  |
| Vector control                         | Aerobic: - - - - - - |  |
|                                        | Anaerobic: - - - - - - |  |

<sup>a</sup> Anaerocult A (Merck) was used for anaerobic culture.

<sup>b</sup> After four passages on solid medium. BA, Columbia blood agar. The intensity of the adherence was classified as follows: ++++, numerous single bacteria (>100) and/or clusters (5 to 10) in each microscopic field; ++, several single bacteria (10 to 20) and/or bacterial clusters (2 to 3) in each field; +, single bacteria (5 to 20) in each field; -, no adhering bacteria.

<sup>c</sup> Duration of exposure of cells to bacteria. The longest time intervals for which no adherence of the vector control strain was observed were used.

DISCUSSION

SF EHEC O157:NM strains are the second most common cause of sporadic HUS in Germany (9, 22) and are emerging pathogens in other countries (5, 7, 17, 30, 35, 40; http://www.eurosurveillance.org/ew/2006/060601.asp#2). Multiple differences in the features of infections involving SF EHEC O157:NM and EHEC O157:H7 (22, 30) point to differences in the nature of the reservoirs, vehicles for transmission, and virulence factors. Currently known differences in putative virulence factors include the presence in SF EHEC O157:NM of a complete efa1 gene (25), which encodes the EHEC factor for adherence (Efa1) (39) or lymphostatin (32), but the absence of the gene clusters encoding tellurite resistance (ter) (8, 42) and urease (ure) (19, 41). Moreover, SF EHEC O157:NM possesses a mosaic genomic island composed of segments of the Shigella resistance locus and the E. coli O157:H7 strain EDL933 genome (27), which is absent from EHEC O157:H7 (27). Also, most SF EHEC O157:NM strains possess the cdi-V cluster, encoding a novel member of the cytotoxic distending toxin family (26), which is only rarely found in E. coli O157:H7 (20). The large plasmid of SF EHEC O157:NM lacks espP and katP (13), which encode the serine protease EspP and catalase-peroxidase, respectively, on pO157 of EHEC O157:H7 (14), but contains a unique sfp gene cluster that mediates the expression of fimbriae when cloned into an E. coli laboratory strain (12). However, Sfp fimbriae have not been demonstrated in wild-type SF EHEC O157:NM (12).

Our data demonstrate a strong influence of growth conditions on the expression of Sfp fimbriae. The anaerobic induction of Sfp fimbriae in wild-type SF. coli O157:NM strains correlated closely with an increased ability to adhere to Caco-2 and HCT-8 cells, suggesting that Sfp fimbriae may contribute to the adherence of these pathogens to human colonic epithelial cells during infection. The finding of anaerobic upregulation of Sfp expression on media simulating the environments of the colon and ileum, where EHEC presumably adheres during human infection (15), supports this conclusion. Taken together, our data show that in SF. coli O157:NM the expression of Sfp fimbriae responds to the needs of the pathogen at the site of the infection. We have also confirmed the findings of Brunder et al. (12) that Sfp fimbriae in such strains are not expressed under standard laboratory conditions. Nevertheless, the inducibility of expression of Sfp fimbriae under particular environmental conditions demonstrates that the sfp cluster in wild-type SF. coli O157:NM strains is functional, in contrast to several fimbrial gene clusters in EHEC O157:H7 strains that apparently do not encode functional fimbriae (36, 48).

Several transcription factors control gene expression in response to key cues, such as oxygen availability (28, 31, 43, 46, 47, 54). These factors either act operon-specifically or are global regulators, coordinating the expression of numerous promoters in response to the availability of oxygen (28, 31, 43,
involved in the regulation of the gastrointestinal tract (traditionally called quorum sensing) is a
intra- and interspecies communication among bacteria in the 46, 47, 54). Moreover, it has become increasingly clear that
transitions from aerobic to anaerobic environments, or vice versa, involve changes in a large number of
genes, or the type III secretion system (4, 16, 21, 24, 46). In EHEC
O157:H7, the most common EHEC serotype associated with human disease (30, 51), upregulation of numerous putative virulence or fitness genes under anaerobic conditions has been reported (4, 16, 21); these include the stx2 gene, encoding Shiga
A. HCT-8 cells

![Image](Image1)

B. Caco-2 cells

![Image](Image2)

FIG. 3. Adherence of SF EHEC O157:NM strain 493/89 grown aerobically (panels 3) or anaerobically (panels 4) to HCT-8 (A) and Caco-2 (B) cells compared with the adherence of HB101/pSFO157-

maximal virulence factor induction at the site of the infection

sfp

E. coli

m.

/H9262

Escherichia coli

vaporization

ins, or the type III secretion system (4, 16, 21, 24, 46). In EHEC

plasmid

E11 (panels 2) and the vector control strain HB101/pBluescript II

KS(+)(panels 1). Bars represent 10 μm.

46, 47, 54). Moreover, it has become increasingly clear that
intra- and interspecies communication among bacteria in the
gastrointestinal tract (traditionally called quorum sensing) is a
common mechanism of gene regulation in pathogenic bacteria
(49, 50). Studies are under way to determine the mechanisms
involved in the regulation of the sfp fimbrial gene cluster.

By responding to environmental signals via global gene reg-
ulators, bacteria adapt their phenotypes accordingly to ensure
maximal virulence factor induction at the site of the infection
(23, 33, 37, 45, 46, 53, 54). E. coli can grow in the presence and
absence of oxygen and thus can survive and multiply following
excretion from its oxygen-starved colonic niche into an exter-
nal aerobic environment. The ability to adapt to such changes
is controlled by alterations in gene expression (24). Specifically,
transitions from aerobic to anaerobic environments, or
vice versa, involve changes in a large number of E. coli genes,
including those participating in different metabolic pathways,
but also virulence genes such as those encoding adhesins, tox-
in, and the type III secretion system (4, 16, 21, 24, 46). In EHEC

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