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Characterization of F107 Fimbriae of *Escherichia coli* 107/86, Which Causes Edema Disease in Pigs, and Nucleotide Sequence of the F107 Major Fimbrial Subunit Gene, *fedA*

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F107 fimbriae were isolated and purified from edema disease strain 107/86 of *Escherichia coli*. Plasmid pIH120 was constructed, which contains the gene cluster that codes for adhesive F107 fimbriae. The major fimbrial subunit gene, *fedA*, was sequenced. An open reading frame that codes for a protein with 170 amino acids, including a 21-amino-acid signal peptide, was found. The protein without the signal sequence has a calculated molecular mass of 15,099 Da. Construction of a nonsense mutation in the open reading frame of *fedA* abolished both fimbrial expression and the capacity to adhere to isolated porcine intestinal villi. In a screening of 28 reference edema disease strains and isolates from clinically ill piglets, *fedA* was detected in 24 cases (85.7%). In 20 (83.3%) of these 24 strains, *fedA* was found in association with Shiga-like toxin II variant genes, coding for the toxin that is characteristic for edema disease strains of *E. coli*. The fimbrial subunit gene was not detected in enterotoxigenic *E. coli* strains. Because of the capacity of *E. coli* HB101(pIH120) transformants to adhere to isolated porcine intestinal villi, the high prevalence of *fedA* in edema disease strains, and the high correlation with the Shiga-like toxin II variant toxin-encoding genes, we suggest that F107 fimbriae are an important virulence factor in edema disease strains of *E. coli*.

Pathogenic *Escherichia coli* bacteria possess one or more virulence factors that, when lost or mutated, result in a decreased capability of the germ to cause disease (1). Colonization factors that enable enteropathogenic bacteria to adhere to intestinal cells and enterotoxins that cause the intact small intestinal mucosa to secrete fluid are some of the best known virulence factors in enterotoxigenic *E. coli* (ETEC) (19, 38).

Edema disease or *E. coli* enterotoxemia occurs in pigs at the age of weaning after infection with *E. coli* strains belonging to certain serotypes (39, 49, 51). The toxin that the bacteria produce in the digestive tract (Shiga-like toxin II variant [SLT-IIv]) is absorbed into the circulation and causes lesions in the vasculature of the intestine, subcutis, and brain. Thus, the typical symptoms of the disease are subcutaneous edema of the eyelids and neurological signs, notably, ataxia, convulsions, and paralysis (9, 14, 29, 31, 33, 40). SLT-IIv is chemically, functionally, and genetically characterized, and its role in the onset of edema disease is clearly established (8, 13, 14, 17, 27, 28, 54).

After oral inoculation of pigs with an edema disease strain of *E. coli*, Smith and Halls (50) found significantly more bacteria in scrapings from the wall of the intestine than in the contents, suggesting that the bacteria adhered to the enterocytes. In experimentally infected pigs, attachment of *E. coli* bacteria to the epithelial cells of the mid-jejunum and ileum was demonstrated (6, 33). Electron microscopic studies showed both continuous layers of bacteria and microcolonies of various sizes along the brush borders. Preferentially, the tips of the villi were colonized, with only a few bacteria being demonstrated in the crypts. The bacteria were in close contact with the microvilli, which seemed morphologically normal (6). An electron-lucent zone was described around the adhering bacteria (33).

Recently, edema disease strain 107/86 of *E. coli* was isolated (5). As was shown by electron microscopy, bacteria of strain 107/86 expressed fimbriae after growth on blood agar. Furthermore, the bacteria attached strongly in vitro to isolated porcine brush border fragments. Also, fimbriate bacteria were demonstrated by indirect immunofluorescence in the intestinal contents of pigs infected with strain 107/86. For these reasons, it was concluded that the bacterial adhesion was mediated by a new type of fimbriae, named F107 (5).

In this report, we describe the isolation and characterization of F107 fimbriae of edema disease strain 107/86 of *E. coli*. The gene cluster encoding F107 fimbriae was cloned, and the major subunit gene was sequenced. The F107 major fimbrial subunit gene was defined as *fedA*, refering to fimbriae associated with edema disease. We suggest that F107 fimbriae are important virulence factors in edema disease-causing strains.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* 107/86 was described earlier (5). The following bacteria were used as edema disease reference strains (51): E4 (O139:K82), E57 (O138:K81), and E145 (O141:K85a,c). A further 24 isolates from clinically ill piglets belonging to the following serogroups were tested: O138 (n = 2), O139 (n = 9), and O141 (n = 13).

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The following reference ETEC strains were provided by I. and F. Ørskov, Copenhagen, Denmark: *E. coli* B_{AM} (type 1), H10407 (CFAI, F2) E4833 (CS2CS3, F3), E1392 (CS1CS3, F3), E68 (K88ab, F4), G1253 (K88ac, F4), 56/190 (K88ad, F4), B41 (K99, F5; F41), B41 $K99^-$ (F41), 987P (F6), C1212-77 [F7(P)F1C], and C1254-77 [F8(P)]. *E. coli* 25KH09 and 111KH86 were described earlier (4, 25).

The genomic library of strain 107/86 was constructed in *E. coli* DH5α (45). *E. coli* HB101 (7) cells were used in studies evaluating the phenotypic expression of the transformants.

Plasmid pUC18 (55) was used as the cloning vector.

Culture conditions. The edema disease strains of *E. coli* were grown in Trypticase soy broth (Bio Mérieux), and the ETEC strains were grown in LB medium (34). The transformants were plated on solid LB medium containing carbenicillin (100 μg/ml), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (40 μg/ml in dimethylformamide), and isopropyl-β-D-thiogalactopyranoside (IPTG) (0.2 mM in water). For the expression studies, the HB101 transformants were grown on Iso-Sensitest agar (Oxoid) supplemented with carbenicillin. All incubations were at 37°C for 20 h.

Fimbrial extraction. Fimbriae were isolated from edema disease strain 107/86 by mechanical disruption, basically as described earlier (21). A 1-liter bacterial culture was centrifuged at 4°C for 10 min at 5,000 × *g*. The bacterial pellet was washed twice in phosphate-buffered saline (PBS; 7 mM phosphate buffer with 3 mM KCl and 128 mM NaCl). The pellet was resuspended in 10 ml of PBS and cooled on ice. After being sheared with an Ultra Turrax (two times for 15 s), the homogenate was dialyzed against water and subsequently precipitated with 20% ammonium sulfate overnight at 4°C. The precipitate was resuspended in 2 ml of PBS. This solution was placed on a Sephacryl S-300 column (Pharmacia; dimensions, 2.5 cm by 1.5 m) and eluted with 100 mM Tris-HCl buffer (pH 7.2) at 40 ml/h under UV monitoring (A_{280}).

Small-scale fimbrial preparations from transformants were obtained by thermoelution (36). In this way, a large number of cultures could be analyzed for the expression of fimbriae. Approximately 4×10^{10} bacteria were centrifuged and resuspended in 1 ml of PBS. This bacterial suspension was heated for 25 min at 65°C and subsequently centrifuged. Of the supernatant, 5 μl was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Preparation of specific antiserum. Rabbits were injected subcutaneously between the toes with an emulsion that contained approximately 250 μg of purified 107/86 fimbrial extract in 1 ml of PBS and 1 ml of complete Freund adjuvant (vol/vol). Two boosters with the same quantity of protein in incomplete Freund adjuvant were given 14 and 28 days after the primary inoculation. Blood was taken 14 days after the last injection.

The antiserum for use in the cloning procedures was absorbed with a culture of strain DH5α(pUC18) to reduce nonspecific reactions in the immune staining. For this purpose, the antiserum was incubated twice for 4 h at 4°C with approximately 2×10^{11} bacteria of strain DH5α(pUC18). In the same way, F107 antiserum was absorbed with HB101(pUC18) for use in Western blotting (immunoblotting) and in immunoelectron microscopy.

Protein and DNA electrophoresis. Fimbrial extracts were analyzed by vertical SDS-PAGE as described previously (22), using the Mini Protean II Dual Slab Cell system from Bio-Rad.

All DNA separations were done by a horizontal electrophoresis system supplied by Bio-Rad. Gels consisted of 0.7% agarose in Tris-borate buffer (30).

Colony blot, Western blot, and immune staining. To screen the genomic library in the colony blot, 2 μl of a culture representing approximately 10^9 transformants was spotted on a nitrocellulose membrane and allowed to dry. Cultures of strain DH5α(pUC18) and of strain 107/86 were spotted on the same membrane as negative and positive controls, respectively.

Western blotting was performed by the technique of Towbin et al. (52). Fimbrial extracts were loaded on SDS-polyacrylamide gels and blotted on a nitrocellulose membrane. After being washed for 15 min with PBS supplemented with 1% ovalbumin, the nitrocellulose membrane was incubated overnight at 4°C with the F107 antiserum diluted 1:200 in PBS containing 1% ovalbumin and 0.2% Triton X-100. Then the membrane was rinsed with 0.2% Triton X-100 in PBS and incubated for 2 h in a solution of PBS containing 1% ovalbumin and 0.2% Triton X-100 and supplemented with goat anti-rabbit peroxidase conjugate (Sigma) at a dilution of 1:1,000. After a final wash in PBS, the nitrocellulose membrane was placed in a solution containing 0.75 mg of 4-chloro-1-naphthol (Sigma) per ml, 0.02% H_2O_2, and 25% methanol in PBS.

Enzymes. Restriction enzymes, T4 DNA ligase, calf intestinal phosphatase, and T4 polynucleotide kinase were purchased from Boehringer Mannheim Biochemicals, and the incubation buffers were as specified by the supplier. Trypsin was a product of Sigma.

Construction of a genomic library. Total genomic DNA was prepared by the method of Dhaese et al. (12). The DNA was partially digested with *Sau*3A (1 U of *Sau*3A per 37.5 μg of DNA) and separated on a sucrose gradient. The fractions containing DNA fragments of approximately 10 to 15 kb were precipitated with ethanol and ligated with *Bam*HI-digested and phosphatase-treated cloning vector pUC18 (30). Recombinants were transformed in DH5α competent cells and selected on LB agar plates containing carbenicillin, IPTG, and X-Gal. White colonies were transferred to solid LB agar plates and subsequently blotted on nitrocellulose membranes. The genomic library was screened for F107 expression in an colony blot with absorbed F107 antiserum.

Electron microscopy. *E. coli* bacteria grown in Trypticase soy broth were adsorbed on carbon-coated Parlodion films supporting 300-mesh copper grids, stained with 0.25 to 1% Na-phosphotungstic acid (pH 6.6) for 20 to 40 s, and immediately examined.

Immunoelectron microscopy was done as described earlier (25).

Adhesion assays. Porcine intestinal villi were prepared and used by the technique of Girardeau (15). The villi were prepared from just-weaned piglets susceptible to the disease. Adhesion was checked with phase-contrast microscopy (×600).

For inhibition studies, α-methyl-D-mannoside, known to inhibit adhesion by type 1 fimbriae (42, 47), was used at a final concentration of 100 mM.

Amino acid sequence analysis. A sample of nonpurified fimbrial extract of strain 107/86 containing 250 μg of protein was precipitated with trichloroacetic acid before being loaded on a vertical discontinuous SDS-polyacrylamide gel. The separating gel was 20% (wt/vol) acrylamide and 0.13% bisacrylamide (pH 8.8). Electrophoresis and blotting on polyvinylidene difluoride (44) membranes, in situ proteolysis with trypsin, peptide purification by reverse-phase high-

TABLE 1. Primer sequences, localizations, and expected sizes of the amplified PCR products

Primer	Oligonucleotide sequence	Location within gene (nucleotides)	Size of amplified product (bp)	Reference or source
FedA 1	GTGAAAAGACTAGTGTTTATTTC	160–182		
FedA 2	CTTGTAAGTAACCGCGTAAGC	649–669	510	This study
SLT-IIAv 1	CCACCAGGAAGTTATATTTCC	403–423		
SLT-IIAv 2	TTCACCAGTTGTATATAAAGA	1141–1161	759	17
SLT-IIBv 1	ATGAAGAAGATGTTTATAGCG	1177–1197		
SLT-IIBv 2	GTTAAACTTCACCTGGGCAAAG	1416–1437	261	17

pressure liquid chromatography, and amino acid sequencing were performed as described previously (3).

Synthesis of oligonucleotide probe. The oligonucleotide derived from the amino acid sequence of the fimbrial protein was synthesized on an Applied Biosystems PCR-MATE DNA Synthesizer (model 391). In each triplet, deoxyinosine was used as the third nucleotide of the codon. The oligonucleotide was purified by ethanol precipitation.

Labeling of the DNA fragments and Southern blot hybridizations. The synthetic DNA consisting of 41 nucleotides was labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase and was used as a probe in hybridization experiments (30). The 0.9-kb *Pst*I-*Pst*I fragment was labeled by using the Multiprime DNA Labeling System (Amersham).

Hybridization experiments with the 41-base oligonucleotide as a probe were done at 40°C in a hybridization mixture containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5% Denhardt's solution, and 0.5% SDS (30). The nylon membrane was subsequently washed with 2× SSC at 40°C.

Hybridization with the 0.9-kb *Pst*I-*Pst*I fragment was performed at 65°C in a hybridization mixture containing 6× SSC, 5% Denhardt's solution, and 0.5% SDS. Washing was done with 0.1× SSC at 65°C.

Nested deletions and sequencing reactions. Nested deletions of clone pIH120 were obtained by the Erase-a-Base System supplied by Promega. The DNA was treated with acid phenol as recommended by the supplier. The digestions were performed at 33°C, digesting approximately 300 nucleotides per min.

Sequencing was done by the dideoxy termination reaction of Sanger et al. (48). Sequenase (United States Biochemical Corp.) and Deaza T7 Sequencing Mixes (Pharmacia) were used. Both the universal and reverse sequencing primers of pUC were used. The nucleotide sequence was analyzed by using the computer software The Gene Construction Kit (Textco, Inc.).

PCR. The GeneAmp DNA Amplification reagent kit with AmpliTaq DNA polymerase (Perkin-Elmer Cetus) was used. The polymerase chain reaction (PCR) conditions were as described by the manufacturer, but in a total reaction volume of 50 μl. As a DNA template, a suspension was used that contained approximately 5×10^6 bacteria and that was boiled for 10 min. The PCR conditions used were as follows: 25 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 2 min of extension at 72°C. The characteristics of the primers used are described in Table 1.

Mutagenesis with *Xba*I linker. Clone pIH100 was constructed by digestion of clone pIH120 with *Xba*I and then treatment with Klenow enzyme and subsequent self-ligation. In this way, the unique *Xba*I site of the pUC18 cloning site was removed. By digestion with restriction enzymes that recognized only unique sites in pIH100 and then treatment with T4 DNA polymerase or Klenow enzyme, blunt-end linear DNA fragments were generated (30). After ligation with the nonphosphorylated *Xba*I linker (dCTAGTCTA GACTAG; New England Biolabs), the DNA was digested with *Xba*I and self-ligated.

Bacterial extract. A bacterial pellet of approximately 10^{10} cells was resuspended in 1 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with 0.1% SDS. After being boiled for 15 min and centrifuged, 5 μl of the supernatant was analyzed on a protein gel and in a Western blot.

Nucleotide sequence accession numbers. The nucleotide sequence data reported here have been submitted to GenBank and assigned the accession number M61713.

RESULTS

Isolation of the F107 fimbriae. Surface structures of bacteria of strain 107/86 were detached by the technique described by Korhonen et al. (21). On SDS-PAGE, the crude bacterial extract showed one major protein band of 15 kDa (Fig. 1, lane 1). This preparation was further purified by 20% ammonium sulfate precipitation and gel filtration on Sephacryl S-300. The protein-rich fraction that eluted with the void volume of the column contained the 15-kDa protein (Fig. 1, lane 2). The elution pattern and the protein gel suggested that the extract of strain 107/86 contained a high-molecular-weight polymer, composed of subunits of 15 kDa.

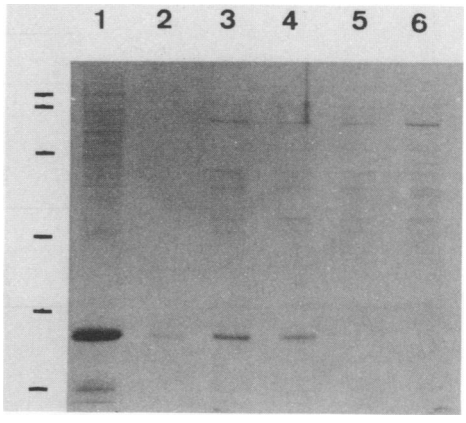

FIG. 1. Protein gel stained with Coomassie blue, representing the crude fimbrial extract of *E. coli* 107/86 (lane 1), its purified extract (lane 2), and the fimbrial preparations obtained by thermoelution of HB101(pIH2) (lane 3), HB101(pIH120) (lane 4), HB101 (pIH123) (lane 5), and HB101(pUC18) (lane 6). Plasmids pIH2 and pIH120 code for nonadhering and adhering F107 fimbriae, respectively. Construct pIH123 contains an insertion mutation in gene *fedA* of construct pIH120. The molecular size markers on the left represent 94, 67, 43, 30, 20.1, and 14.4 kDa.

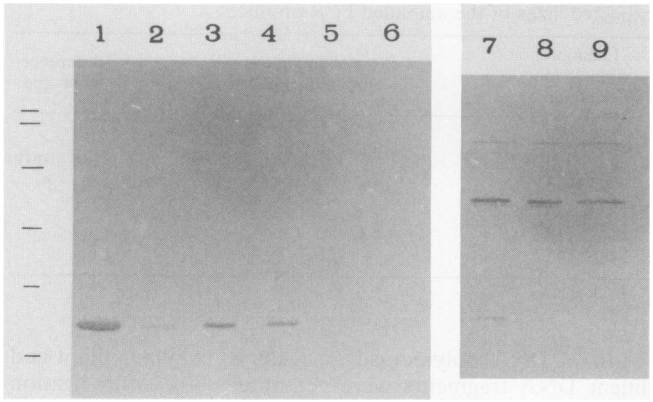

FIG. 2. Immunoblot analysis with F107 antiserum. The membrane containing lanes 1 to 6 was immunostained with F107 antiserum absorbed with HB101(pUC18); the membrane with lanes 7 to 9 was stained with the nonabsorbed F107 antiserum. The samples in lanes 1 to 6 are the same as in Fig. 1. Lanes 7 to 9 represent the bacterial lysates of HB101(pIH120) (lane 7), HB101(pIH123) (lane 8), and HB101(pUC18) (lane 9). The various constructs and the sizes of the molecular size markers on the left are described in the legend to Fig. 1.

Antiserum was prepared against the purified extract of strain 107/86. This serum reacted in a Western blot specifically with the 15-kDa protein band (Fig. 2, lanes 1 and 2). With preimmune antiserum, no protein band was detected (data not shown).

Cloning of the F107 fimbrial gene cluster and phenotypic expression of the transformants. A genomic library of *E. coli* 107/86 was constructed in cloning vector pUC18. The transformants in DH5α were screened with F107-specific antiserum in a colony blot. Two independent clones were isolated, pIH2 and pIH120. A restriction map is given in Fig. 3. Plasmids pIH2 and pIH120 have inserts of 6.8 and 7.3 kb, respectively. The clones overlap for approximately 5.8 kb (Fig. 3).

Plasmids pIH2 and pIH120 were transformed in HB101 cells and subsequently examined for their expression of F107 fimbriae. By electron microscopy, fimbriae with a diameter of approximately 5 nm were seen (Fig. 4). A small-scale isolation of fimbriae from these transformants was prepared by thermoelution. The eluates were analyzed on a protein gel and showed a protein band of about 15 kDa, representing the F107 subunit (Fig. 1, lanes 3 and 4). This major fimbrial protein was also recognized in a Western blot (Fig. 2, lanes 3 and 4). By immunoelectron microscopy with F107 antiserum, specific binding of gold particles was seen, both on the bacterial surface and on structures that are perpendicular to the wall of the bacteria (Fig. 5).

Strain 107/86 and transformants HB101(pIH2) and HB101 (pIH120) were tested in the in vitro adhesion assay for their ability to attach to isolated intestinal porcine villi. Bacteria of strain 107/86 and of transformant HB101(pIH120) attached specifically, and also attached in the presence of mannose. Transformant HB101(pIH2) did not attach to the isolated villi.

Localization of the subunit gene. To localize the F107 fimbrial subunit gene in the cloned sequences, we prepared a specific oligonucleotide. For this purpose, the amino acid sequence of an internal peptide of the purified F107 subunit was determined. The following 14-amino-acid sequence was obtained: Gly-Gly-Gln-Thr-Pro-Gly-Asp-Phe-Gln-Gly-Ala-Ala-Ala-Tyr. Based on this sequence, the following 41-base oligonucleotide was synthesized: GGI GGI CAI ACI CCI GGI GAI TTI CAI GGI GCI GCI GCI TA. In Southern blot analysis with the 41-base oligomer used as a probe, the 0.9-kb *Pst*I-*Pst*I and the 2.4-kb *Hpa*I-*Hpa*I fragments of pIH2 were marked (Fig. 3). It was concluded that the nucleotide sequence coding for the internal peptide of the F107 subunit is located between the *Hpa*I site at 2.6 kb and the *Pst*I site at 3.1 kb on the restriction map of clone pIH2, or between 1.6 and 2.1 kb in clone pIH120 (Fig. 3).

Nucleotide sequence of the *fedA* gene. Subclones were made that allowed the sequencing of both strands of the region containing the F107 major fimbrial subunit gene, *fedA*. The

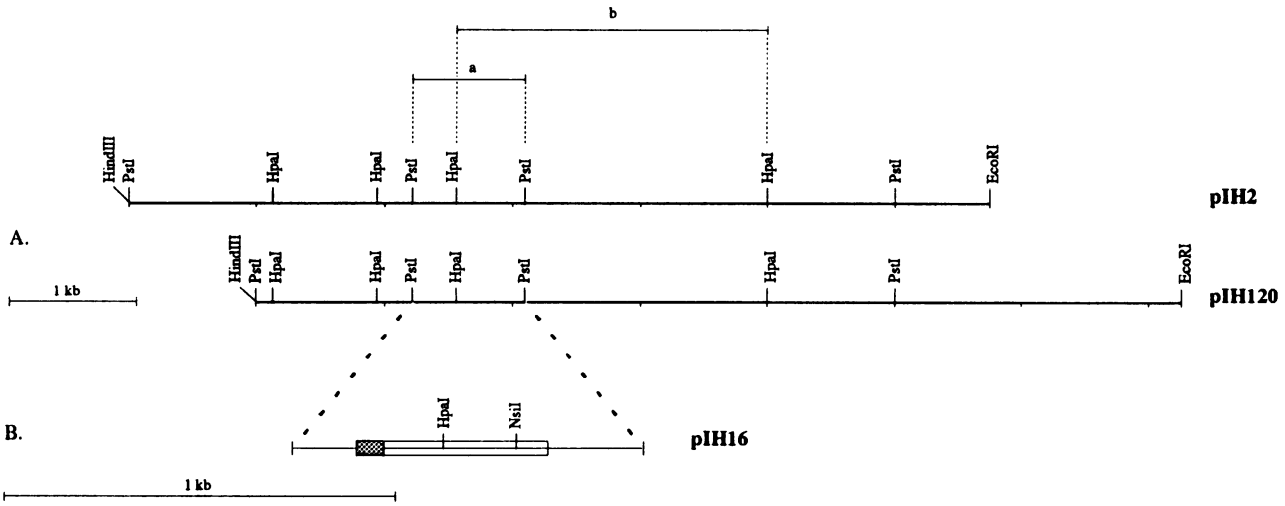

FIG. 3. Alignment of plasmids pIH2 and pIH120 and localization of the F107 structural gene. (A) Restriction map of pIH2 and pIH120 demonstrating their relative positions. The 0.9-kb *Pst*I-*Pst*I (a) and *Hpa*I-*Hpa*I (b) fragments that reacted with the oligonucleotide that was derived from the amino acid sequence of the internal peptide of FedA are schematically indicated above plasmid pIH2. (B) Localization of *fedA* (box) in plasmid pIH16. The hatched part represents the signal sequence of the fimbrial subunit. The *Nsi*I site in which the *Xba*I linker was inserted (resulting in plasmid pIH123) is also indicated.

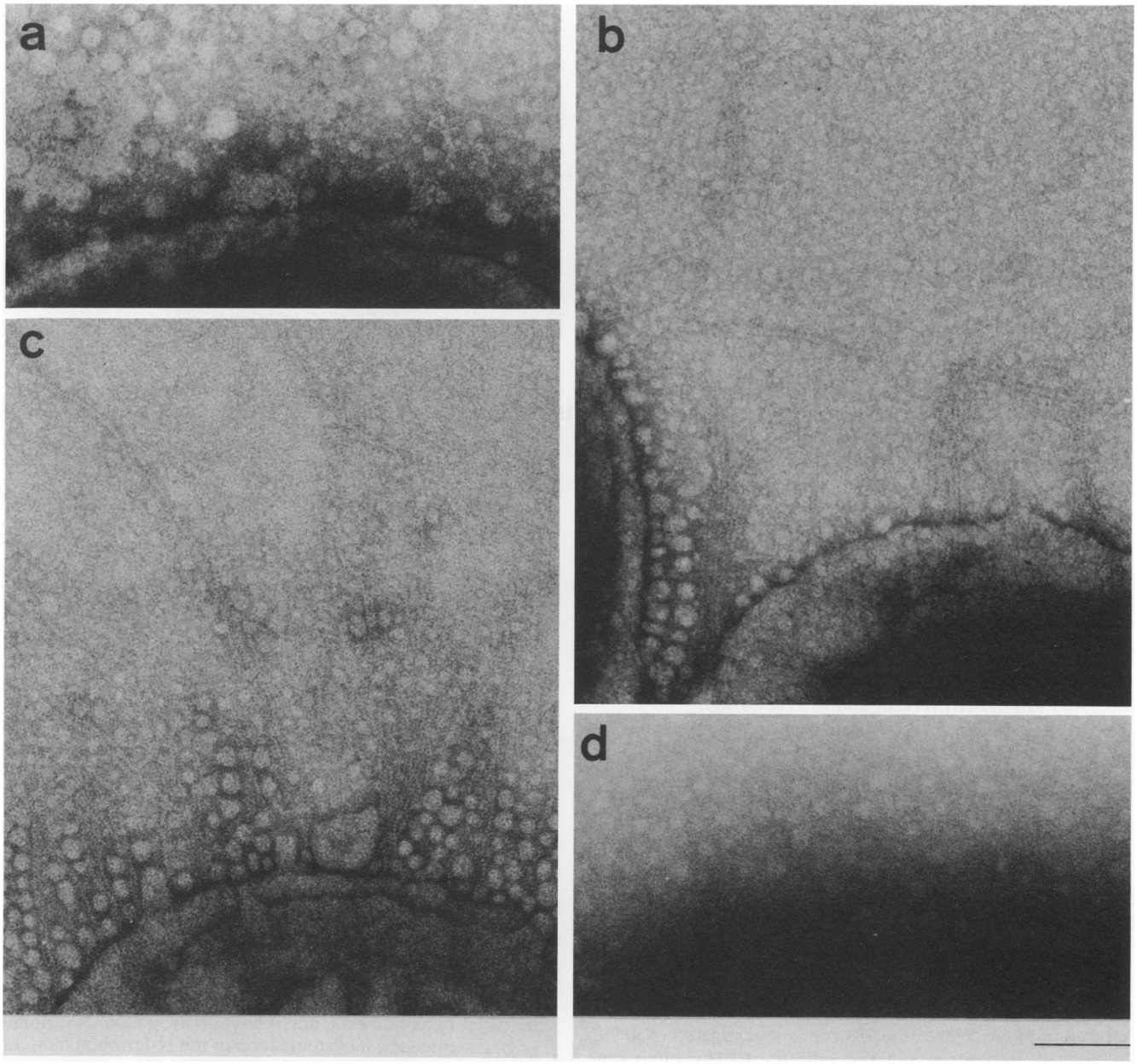

FIG. 4. Electron micrographs of negatively stained *E. coli* 107/86 and three transformants. (a) HB101(pUC18); (b) HB101(pIH2); (c) HB101(pIH120); (d) HB101(p123). The bar represents 0.1 μm.

nucleotide sequence of clone pIH16 that contains the 914-bp *Pst*I-*Pst*I fragment (Fig. 3) is given in Fig. 6.

An open reading frame was found that coded for 170 amino acids, starting with the GTG codon at position 160 of clone pIH16. The 14-amino-acid sequence that was determined from the internal peptide of the fimbrial subunit was found in this open reading frame at positions 613 to 654. Putative promoter sequences, a ribosome-binding site, and a transcription terminator were found as indicated in Fig. 6. A putative signal sequence, comprising 21 amino acids, was found at position 160 to position 222. This amino acid sequence has two positively charged amino acids at the amino terminus and then a stretch of 18 primarily hydrophobic amino acids and ends with alanine-methionine-alanine, which was demonstrated adjacent to the processing site of outer membrane proteins and periplasmic proteins in gram-negative bacteria (43). The remaining amino acid sequence predicts a mature protein of 15,099 Da.

Insertion mutagenesis of clone pIH120. In the unique *Nsi*I site located at 1.7 kb in plasmid pIH120 (Fig. 3), an *Xba*I linker was incorporated. This construct, pIH123, was transformed in HB101 cells and did not code for F107 fimbriae, as was demonstrated by electron microscopy (Fig. 4) and thermoelution (Fig. 1 and 2, lanes 5). In immunoelectron microscopy with the F107 antiserum that was absorbed to HB101(pUC18) bacteria, no linear organization of gold grains was seen, although a number of particles were randomly dispersed over the field (Fig. 5). Bacterial lysates of

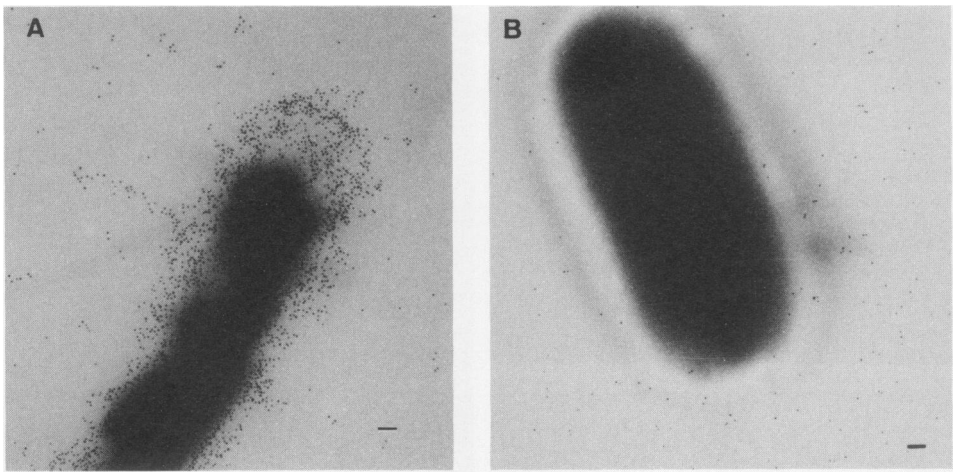

FIG. 5. Immunoelectron microscopy of HB101(pIH120) (a) and HB101(pIH123) (b). The F107 antiserum was absorbed against HB101 (pUC18). The bars represent 0.1 μm.

transformants HB101(pIH120) and HB101(pIH123) were prepared and analyzed in Western blots with F107 antiserum. In the sample prepared from HB101(pIH123), the 15-kDa subunit was not detected, as opposed to HB101 (pIH120), demonstrating that the F107 subunit was no longer expressed (Fig. 2, lanes 7 and 8). HB101(pIH123) was not able to adhere in the in vitro test to isolated porcine villi, as opposed to HB101(pIH120) and *E. coli* 107/86.

Prevalence of the F107 subunit gene and association with SLT-IIv genes. To detect *fedA* in the genomes of enterotoxigenic and edema disease strains of *E. coli*, we used the 914-bp *Pst*I-*Pst*I fragment as a probe in Southern blots. Also, *fedA*-specific primers were synthesized for use in PCR (Table 1). Restriction enzyme-digested total genomic DNA of ETEC strains expressing type 1, CFA1, CFA2 (CS1CS3 and CS2CS3), K88 (ab, ac, and ad), K99, 987P, F41, F7(P)F1C, F8(P), F17, and F111 fimbriae did not hybridize with the F107 structural gene or show the 510-bp band on an agarose gel (data not shown). However, in reference edema disease strains and in isolates from clinical cases of edema disease, including *E. coli* 107/86, the *fedA* gene was demonstrated in 24 (85.7%) of the 28 strains investigated (Table 2).

To evaluate their association with the F107 subunit gene, we looked for *slt-IIvA* and *slt-IIvB* in the same edema disease strains of *E. coli* by PCR. As indicated in Table 2, among the 24 *fedA*-positive strains, we found 20 strains (83.3%) also bearing the SLT-IIv genes.

Amino acid sequence homology. The deduced amino acid sequence of the F107 subunit was compared with those of the fimbrial subunits of K99 (46), K88ab (minor subunit) (37), 987P (11), type 1 (20), Pap (2), and F17 (26) fimbriae (Fig. 7). Taking into account the functional similarity among the amino acids phenylalanine, tryptophan, and tyrosine, between threonine and serine, and among histidine, lysine, and arginine (10), the most homology was found to the sequence of the K99 fimbrial subunit: 60 amino acids could be aligned with the amino acid sequence of the F107 fimbrial protein. Homology to the fimbrial subunits of K88, 987P, type 1, Pap, and F17 was less pronounced: only 46, 34, 46, 42, and 35 functionally similar amino acids, respectively, were aligned.

DISCUSSION

E. coli 107/86 was the first edema disease strain reported that expressed fimbriae in vitro (5, 5a). In this report, we describe the isolation, characterization, and cloning of the F107 fimbriae. Both clones pIH2 and pIH120 code for fimbriae that have a diameter of approximately 5 nm and that morphologically resemble those of *E. coli* 107/86. The filaments are equally well distributed along the surface of the bacteria. In fimbrial preparations of strain 107/86 and of transformants HB101(pIH2) and HB101(pIH120), the subunit has a molecular mass of 15 kDa. The antiserum that was raised against the fimbrial preparation of strain 107/86 specifically stained the structural protein in Western blots. Therefore, we conclude that the genes that are necessary for the expression of the F107 fimbriae are present in both plasmids pIH2 and pIH120.

The F107 subunit gene was identified with an oligonucleotide that was derived from the amino acid sequence of an internal peptide of the structural protein. The deduced amino acid sequence of FedA has characteristics in common with most *E. coli* fimbrial subunits (41): the protein has a size between 14 and 22 kDa, and it has a hydrophobic N terminus (signal sequence), a cysteine loop in the N-terminal half, and a penultimate tyrosine residue at its C end.

In its N- and C-terminal ends, the amino acid sequence of FedA shows similarity with the subunits of K88, K99, type 1, Pap, and F17 fimbriae. It is suggested that these conserved regions are involved in the structural characteristics of the fimbriae or in the direction of the proteins to their ultimate locations (20, 37). The C-terminal part of the FedA protein is hydrophobic (data not shown). In Pap fimbriae, the hydrophobic carboxy-terminal part of the structural protein was thought to be involved in subunit-subunit interaction, membrane embedding, or both (41). The greatest amino acid sequence homology of FedA was found to the K99 fimbrial subunit. The resemblance in primary structure suggests that the fimbrial subunits of F107 and K99 (that may be present in porcine ETEC strains) evolved from a common ancestral gene.

Although both clones pIH2 and pIH120 code for the proteins that are necessary for the biogenesis of the F107 fimbriae (subunit, transport of the fimbrial proteins through the periplasma and outer membrane, stabilization, and poly-

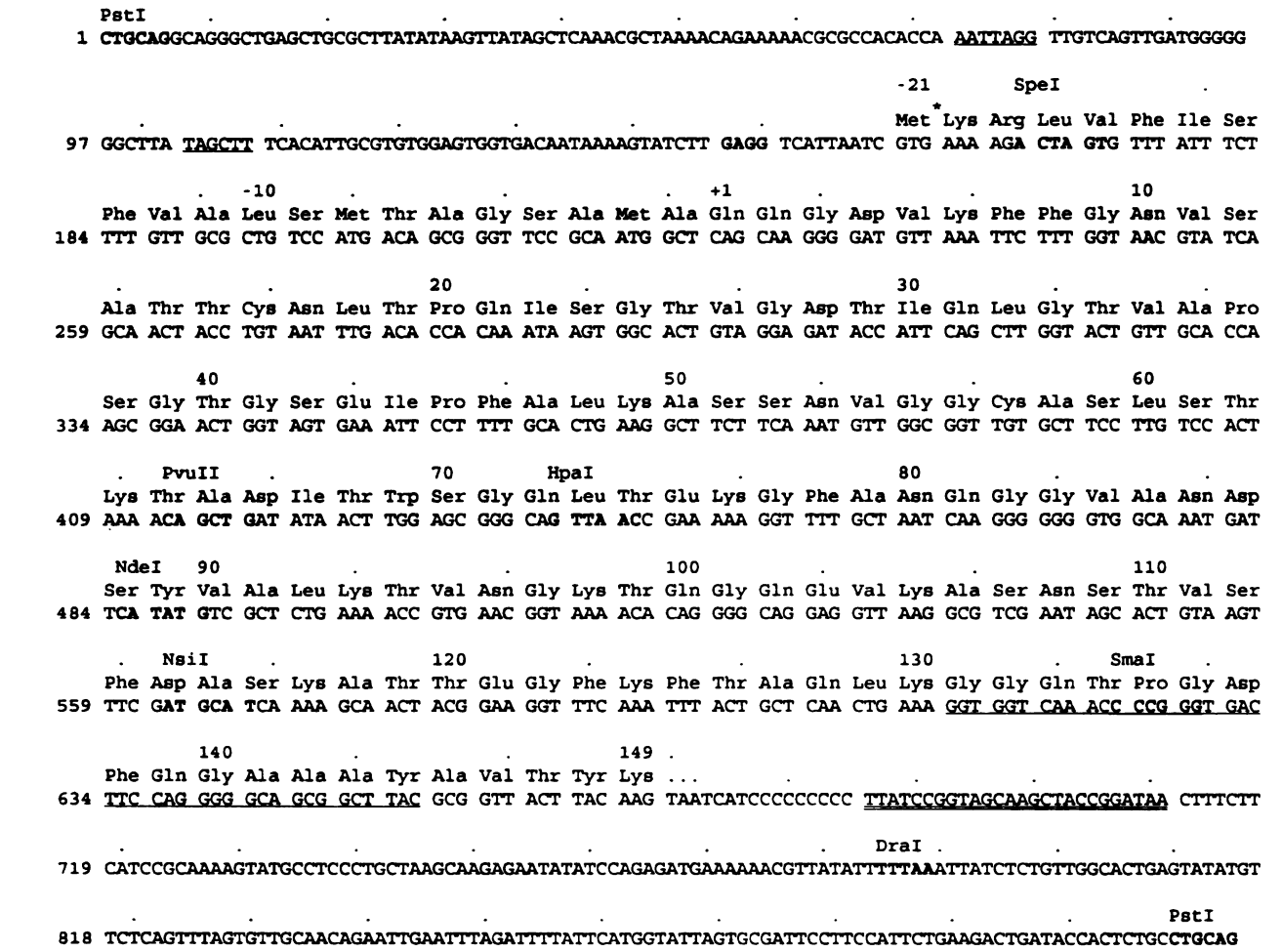

FIG. 6. Nucleotide sequence of plasmid pIH16, containing the *fedA* gene. The amino acid sequence of the predicted polypeptide is shown above the nucleotide sequence. Numbers above each line refer to amino acid positions. The amino acids constituting the putative signal sequence are indicated by negative numbers. The first amino acid of the mature protein is numbered +1. The putative promoter sequences and the DNA sequence encoding the internal peptide are underlined. A putative ribosome-binding site and transcription terminator are indicated in boldface and by double underlining, respectively. *, The GTG initiation codon is translated as formyl-methionine as the first amino acid of the protein sequence (16).

merization), only transformant HB101(pIH120) expressed fimbriae and attached in a mannose-resistant way to isolated porcine villi. It may be concluded that the receptor-recognizing function of the F107 fimbriae is not located on the fimbrial subunit or on the proteins that are involved in the expression of the fimbriae. As with type 1, Pap, and F17 fimbriae, a separate fimbrial protein, the fimbrial adhesin, is likely to bear this function in F107 fimbriae (24, 32, 53).

TABLE 2. Prevalence of *fedA* in reference strains and in clinical isolates of edema disease *E. coli* strains and their association with *slt-IIvA* and *slt-IIvB*

Strain (no. of isolates)	*fedA*$^+$ SLT-IIv$^+$	*fedA*$^+$ SLT-IIv$^-$	*fedA*$^-$ SLT-IIv$^+$	*fedA*$^-$ SLT-IIv$^-$
O138 (n = 3)	1	1	0	1
O139 (n = 11)	6	2	3	0
O141 (n = 14)	13	1	0	0
Total (n = 28)	20	4	3	1

Since the sequences of plasmids pIH2 and pIH120 do not overlap completely, it is thought that the adhesin-coding sequences are not complete in clone pIH2, as opposed to clone pIH120. Therefore, these sequences are situated in the distal half of the gene cluster, downstream of *fedA*. Mutagenesis studies and in vitro transcription-translation studies indeed have identified a 32-kDa protein in this region. Its characterization is under study at the moment.

Insertion inactivation of the *fedA* gene completely abolished subunit expression, and the F107 fimbriae were no longer produced. Moreover, transformant HB101(pIH123) did not attach to isolated porcine intestinal cells. As with type 1 and F107 fimbriae, complete fimbriae apparently are indispensable for the expression of the F107 adhesin (18, 24, 35). In Pap fimbriae, on the contrary, the fimbrial subunit is not necessary for the digalactoside-specific binding (23, 53).

In a limited study, we looked for the prevalence of the F107 structural gene and of the SLT-IIv genes in edema disease reference strains and in clinical isolates. Not all of the investigated strains contained both factors F107 and SLT-IIv. It is conceivable that fimbriae or toxins other than

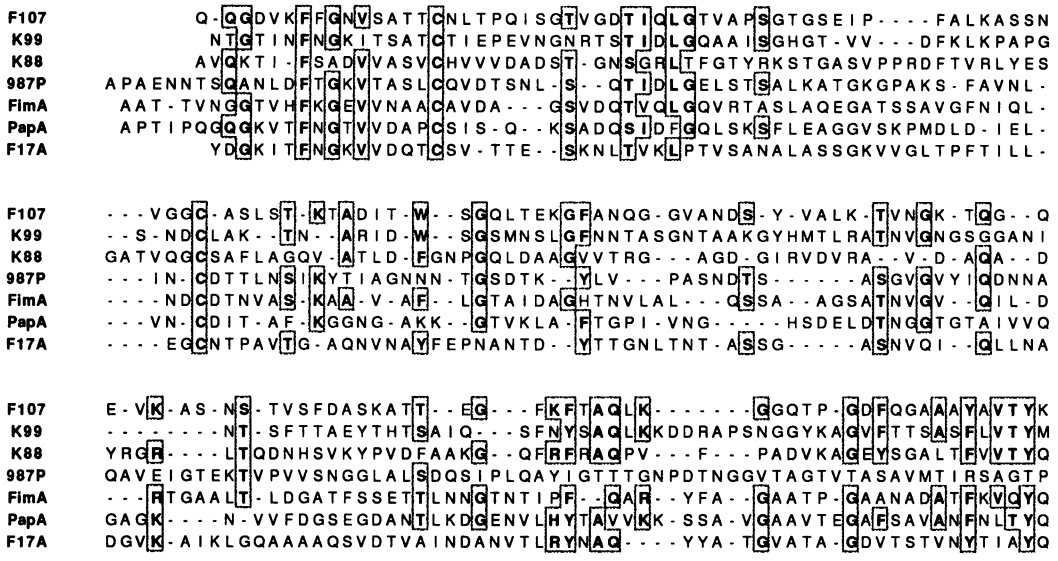

FIG. 7. Alignment of the primary structure of the F107 fimbrial subunit FedA with the amino acid sequences of the K99, K88ab (small subunit), 987P, FimA, PapA, and F17A fimbrial subunits. The amino acid sequence is given in the single-letter code. ST, FWY, and KHR are assumed to be functionally similar (10). Dashes indicate gaps introduced to increase the number of matches. Amino acids that are identical with the F107 protein are boxed when at least four of the compared amino acids occur at a given position.

F107 or SLT-IIv, respectively, will in the future be isolated and characterized in edema disease strains of *E. coli*. Furthermore, the F107-negative or SLT-IIv-negative strains could have lost these factors upon storage. Only infection studies with weaned pigs may elucidate this. Because the majority of the edema disease strains have the *fedA* gene in their genome, and because of the close association with the SLT-IIv genes, we suggest that the adhesive F107 fimbriae are a virulence factor in edema disease strains of *E. coli*.

The characterization of F107 fimbriae of edema disease strain 107/86 is, together with the characterization of the SLT-IIv toxin, an essential breakthrough in the understanding of the pathogenesis of the disease. Further work will concentrate on the genetic organization of the F107 fimbrial gene cluster in general, and on the adhesin gene especially.

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