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Identification of Minor Fimbrial Subunits Involved in Biosynthesis of K88 Fimbriae

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The nucleotide sequences of the genes *faeF*, *faeH*, *faeI*, and *faeJ* encoding K88 minor fimbrial subunits were determined. Analysis of the primary structure of the gene products revealed that all four proteins are synthesized with an amino-terminal signal sequence. The molecular masses of the mature FaeF, FaeH, FaeI, and FaeJ proteins were calculated to be 15,161, 25,461, 24,804, and 25,093 Da, respectively. FaeH, FaeI, and FaeJ showed significant homology with FaeG, the major fimbrial subunit of K88 fimbriae. Mutations in the respective genes were constructed. Analysis of the mutants showed that the minor fimbrial subunits FaeF and FaeH play an essential role in the biogenesis but not in the adhesive properties of the K88 fimbriae. Mutations in *faeI* or *faeJ* had no significant effect on K88 production or adhesive capacity. Specific antisera against FaeF and FaeH were raised by immunization with hybrid Cro-LacZ-FaeF and Cro-LacZ-FaeH proteins. Immunoblotting and immunoelectron microscopy revealed that FaeF and FaeH are located in or along the K88 fimbrial structure.

The genetic determinant for the biosynthesis of K88 fimbriae in porcine enterotoxigenic *Escherichia coli* is located on large, usually nonconjugative plasmids (21). Analysis of the genetic organization of the K88ab gene cluster has revealed that at least six structural genes (*faeC-faeH*, Fig. 1) are involved in the biosynthesis of K88 fimbriae (13, 16). *faeG* encodes the major fimbrial subunit with a molecular mass of 27,540 Da (2). *faeC* encodes a minor component (16,900 Da) predominantly located at the tip of the fimbriae (14, 17). *faeD* encodes an outer membrane protein (82,100 Da) involved in translocation of the fimbrial subunits to the cell surface (11). *faeE* encodes a periplasmic chaperone (24,768 Da) that assists in the folding of fimbrial subunits into an export-competent configuration, protects the fimbrial subunits against hydrolysis by the DegP protease, and prevents their polymerization in the periplasmic space (1). The involvement of *faeF* and *faeH* in the biosynthesis of K88 fimbriae has been demonstrated by mutational inactivation of these genes (16). Mutants lacking either the FaeF or FaeH polypeptide are impaired in the production of fimbriae.

In this study, the nucleotide sequences of the genes encoding FaeF, FaeH, and the hitherto unknown FaeI and FaeJ polypeptides are presented. Mutants defective for these proteins were constructed and used to investigate the

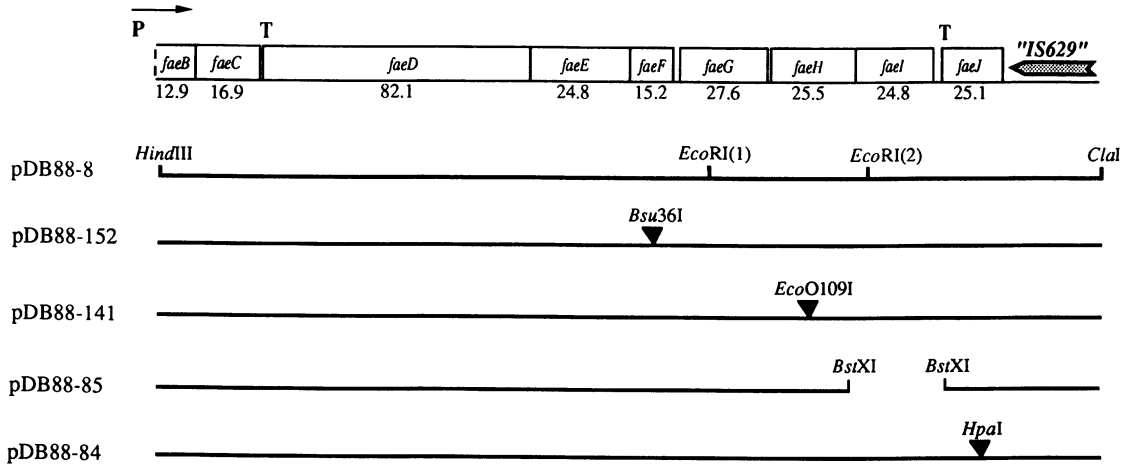

FIG. 1. Genetic and physical maps of the plasmids used in this study. Boxes indicate sizes and locations of the respective genes. Numbers below the boxes refer to the sizes of the mature proteins in kilodaltons. The arrow indicates the direction of transcription. Mutations in *faeF*, *faeH*, *faeI*, and *faeJ* are indicated by the restriction sites used for their construction. P, promoter; T, terminator of transcription.

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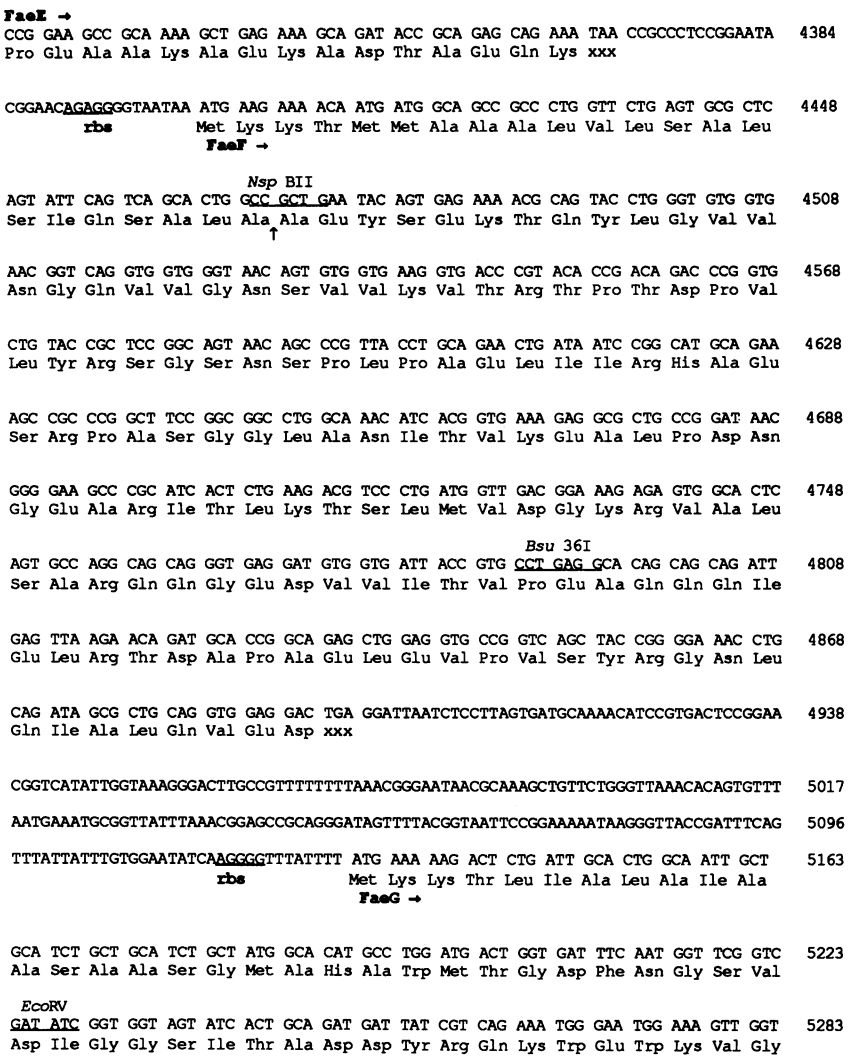

FIG. 2. Nucleotide sequence of the region between bp 4322 and 5283 of the K88 gene cluster, encoding the 3′ end of *faeE*, the complete *faeF* gene, and the 5′ end of *faeG*. The translation of the respective (parts of the) genes is depicted. Stop codons (xxx), the putative ribosome binding site (rbs), and the predicted cleavage site for the signal peptidase (↑) are indicated. The restriction site *Nsp*BII used for the construction of the *cro-lacZ-faeF* fusion and the *Bsu*36I restriction site used for the construction of the stop codon mutation are also indicated.

role of these polypeptides in the biosynthesis and properties of K88 fimbriae.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* K-12 (C600, λ⁻ *tonA21 thr leu-6 thi-1 supE44 lacY-1*^{stable}) was used as a host for the K88-encoding plasmids and their mutant derivatives. *E. coli* JM101 containing plasmid pcI857 was used as a host for the plasmids pEX1, pEX2, and pEX3 and their derivatives (19). *E. coli* JM103 was used as a host for phage M13mp18 and M13mp19 and their derivatives (10). *E. coli* K-12 DS410 was used for the isolation of minicells.

Plasmid pMK077 containing the genes *faeC-faeF* of the K88ac operon was obtained from M. Kehoe (6). For all experiments, strains were grown in Trypticase soy broth except for strain JM103, which was grown in TY medium, (8 g of Bacto Tryptone, 5 g of yeast extract, 5 g of NaCl per liter of distilled water). When appropriate, the media were supplemented with chloramphenicol (40 μg/ml), ampicillin (200 μg/ml), and/or kanamycin (50 μg/ml).

DNA techniques and sequence analysis. All basic recombinant DNA procedures were done essentially as described by Maniatis et al. (8). Nucleotide sequence analysis was done by the dideoxy chain termination method (20) with an automated DNA sequencer (Applied Biosystems 370A) and fluorescent dye-labelled primers. Subclones needed for the analysis of internal DNA fragments were obtained with an Erase-a-base kit (Promega).

Construction of *cro-lacZ* gene fusion. For the preparation of specific polyclonal antisera against FaeF, FaeH, and FaeI, gene fusions between *cro-lac* and the respective genes were constructed by using the pEX cloning vectors (23). The *faeF-cro-lacZ* fusion was constructed by insertion of an *Nsp*BII-*Eco*RV fragment containing nucleotides 4470 to 5226 in the *Sma*I restriction site of pEX1 (see Fig. 2). The fusion protein contained the complete amino acid sequence of the mature FaeF. To obtain a *faeH-cro-lacZ* gene fusion,

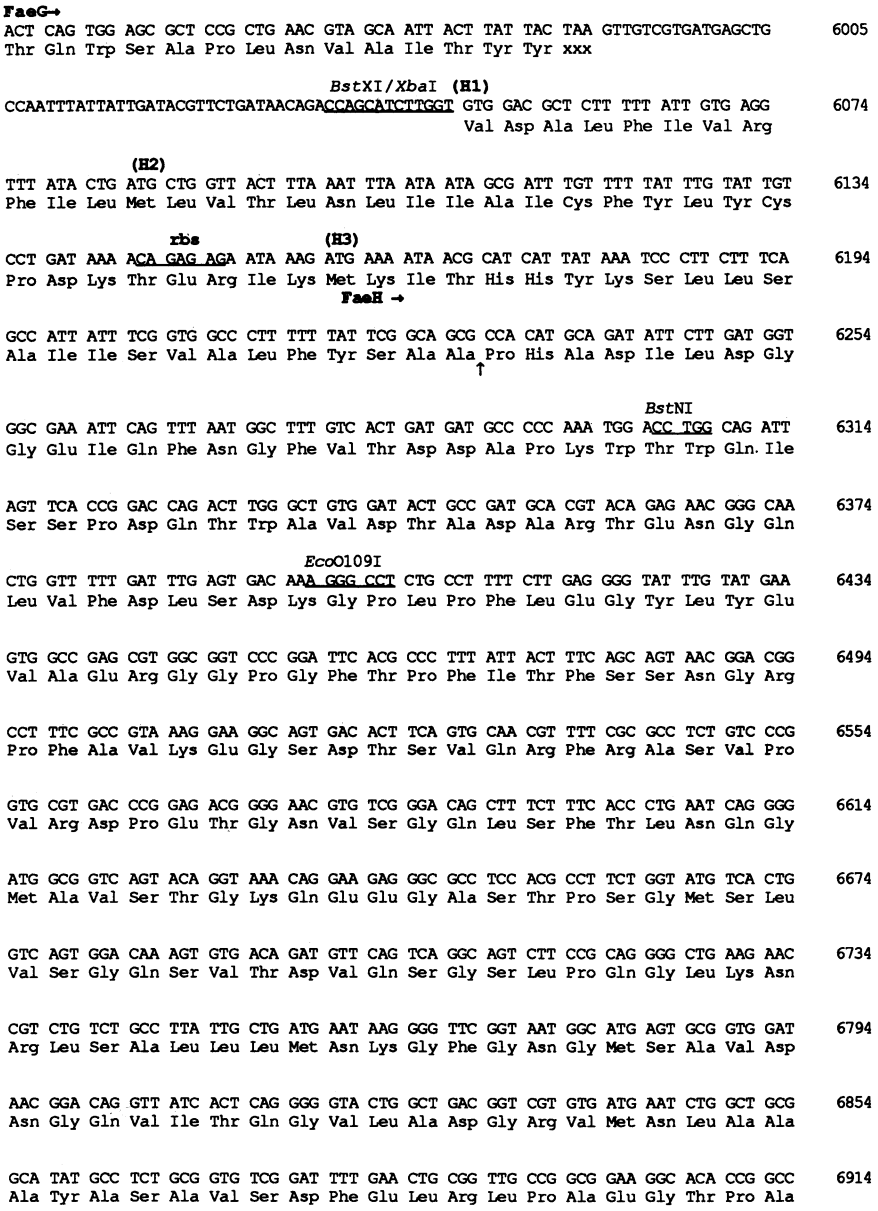

FIG. 3. Nucleotide sequence of the region between bp 5943 and 9446 of the K88 gene cluster, encoding the genes *faeG* (3′ end)-*faeJ* and an IS*629*-like insertion sequence. Translation of the respective *fae* genes and part of a putative transposase, transcribed from the complementary DNA strand, is depicted. Recognition sites for restriction endonucleases used for the construction of mutants and gene fusions are indicated. Stop codons (xxx), putative ribosome binding sites (rbs), the predicted cleavage site for the signal peptidase (↑), and the inverted repeat (I.R.) of IS*629* are indicated.

we inserted the *Bst*NI fragment containing nucleotides 6305 to 7045 (see Fig. 3), after treatment with DNA polymerase I (Klenow fragment), into the *Sma*I restriction site of pEX3. The resulting fusion protein contained the C-terminal 216 amino acid residues of FaeH. The *faeI-cro-lacZ* gene fusion was constructed by insertion of the *Hin*cII fragment encompassing nucleotides 7218 to 8356 (see Fig. 3) into the *Sma*I restriction site of pEX2. The resulting fusion protein contained the C-terminal 178 amino acid residues of FaeI.

The desired fusion proteins were isolated and used for immunization of both Dutch rabbits and Swiss mice as described by Simons et al. (22). The specificity of the sera raised was increased by preadsorption with cell extracts of *E. coli* cells harboring pEX1 as well as *E. coli* cells harboring the appropriate K88 mutant plasmid.

Construction of mutations in *faeF*, *faeH*, *faeI*, and *faeJ*. For the construction of a stop codon mutation in *faeF*, plasmid pDB88-8 was digested with the endonuclease *Bsu*36I, which recognizes a unique site at position 4789 in *faeF* (see Fig. 2), and then a linker composed of the oligonucleotides 5′-TGACTAGTCA-3′ and 5′-TCATGACTAG-3′ was inserted. The resulting plasmid was named pDB88-152. A stop codon mutation in *faeH* was constructed by insertion of a linker into the unique *Eco*O1091 restriction site in pDB88-8 at position 6399 (see Fig. 3). The linker was composed of the oligonucleotides 5′-GGCTCTAGA-3′ and 5′-GCCTCTAGA-

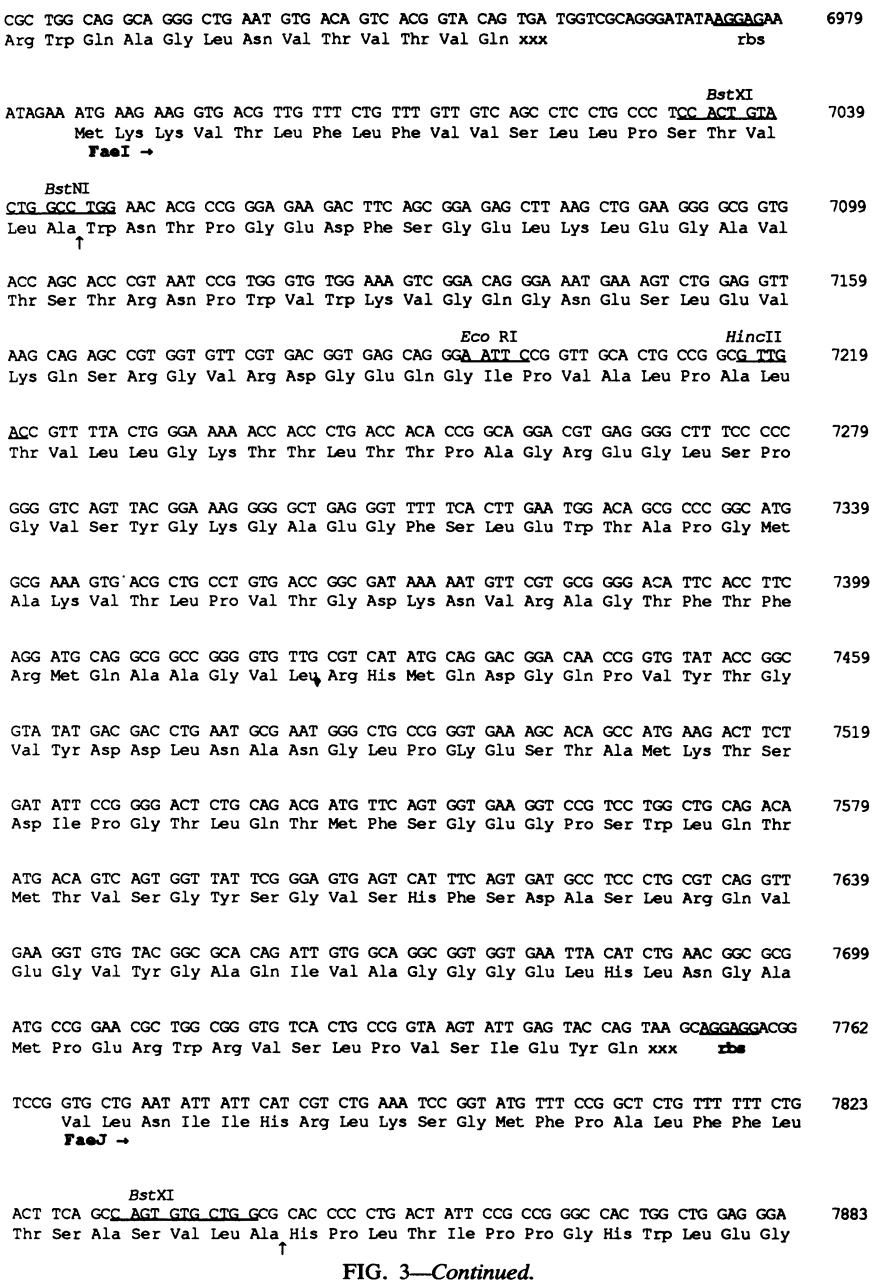

FIG. 3—*Continued.*

3′. The resulting plasmid was named pDB88-141. The deletion mutation in *faeI* was constructed by digesting pDB88-8 with *Bst*XI and religating with T4 DNA ligase, which resulted in removal of the *Bst*XI fragment between nucleotides 7039 and 7838 (see Fig. 3). A frameshift mutation in *faeJ* was constructed by insertion of a *Cla*I linker into the unique *Hpa*I-*Hin*cII restriction site in pDB88-8 at position 8356. The linker was composed of the oligonucleotide 5′-CATCGATG-3′. The resulting plasmid was named pDB88-84.

Minicell analysis. *E. coli* minicells were isolated from overnight cultures of DS410 harboring the required plasmid. Minicells were purified as described previously (13) and labeled with a mixture of [^{3}H]amino acids (50 μCi/ml).

Immunoblotting. Protein samples were separated on 11% polyacrylamide gels essentially as described by Laemmli (7) and then transferred onto nitrocellulose filters. The blots were incubated with the appropriate serum and then incubated with goat anti-mouse or goat anti-rabbit antibodies conjugated to horseradish peroxidase.

ELISA. An enzyme-linked immunosorbent assay (ELISA) was used for the semiquantitative detection of fimbriae produced by *E. coli* cells harboring pDB88-8 or its mutant derivatives (12).

Hemagglutination assay. Binding to chicken erythrocytes was tested in a hemagglutination assay with *E. coli* K-12 cells producing K88ab wild-type or mutant fimbriae. The assay was done essentially as described by Jacobs et al. (4).

Electron microscopy. *E. coli* cells harboring pDB88-8 or one of its mutant derivatives were grown in Trypticase soy broth and collected in the exponential phase of growth.

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ATG GCT GTG GGC GTA ACG GAA CTC AGC GGT ACG CTG TAT GTC CGC GAT GTG TCC TGG CAG    7943
Met Ala Val Gly Val Thr Glu Leu Ser Gly Thr Leu Tyr Val Arg Asp Val Ser Trp Gln

TGG CAG CCC CGC GCT GTG CGG ATG AGC TCT CCT GAT GCA GTG CAG GCT GGC CTG GCA GCA    8003
Trp Gln Pro Arg Ala Val Arg Met Ser Ser Pro Asp Ala Val Gln Ala Gly Leu Ala Ala

GGT AAA GGT GGC ATG GTC AGT GAA AGC CGG AGA GGG CAG GAT TTT TAT ATT CTT GGC GGA    8063
Gly Lys Gly Gly Met Val Ser Glu Ser Arg Arg Gly Gln Asp Phe Tyr Ile Leu Gly Gly

CAT ACC ACA TCA CTG ACA ACT GCC CGT TCG GGG CTG CAG CCG TCG GTG ACA TTA CTT CAG    8123
His Thr Thr Ser Leu Thr Thr Ala Arg Ser Gly Leu Gln Pro Ser Val Thr Leu Leu Gln

GTG GCG CCA TCA TCT CCC CGT ATT GCG GCC CGG GGT GAG CTT GCC CGG GGA CAG GTG CGT    8183
Val Ala Pro Ser Ser Pro Arg Ile Ala Ala Arg Gly Glu Leu Ala Arg Gly Gln Val Arg

TAC GGG GAA ATC ACG TTC ACG CTG CGC CAT CTT CTG GCA TGG CAG GAC AAT ATT ACT GGC    8243
Tyr Gly Glu Ile Thr Phe Thr Leu Arg His Leu Leu Ala Trp Gln Asp Asn Ile Thr Gly

GGT CAG GGC TGG AGC GTG GTC AGC GGA GAG GTG ACG CCG GAG GCC GAA AAG CAG GTG AAA    8303
Gly Gln Gly Trp Ser Val Val Ser Gly Glu Val Thr Pro Glu Ala Glu Lys Gln Val Lys

                                                          HincII
CGC CAG TTA TGG CAG GTG AAC GGC TAT GAA TGG ACC CCG Gac TAT GCC GGG TTA ACC GCG    8363
Arg Gln Leu Trp Gln Val Asn Gly Tyr Glu Trp Thr Pro Asp Tyr Ala Gly Leu Thr Ala

CGT CCT GAT GCG TTT ATT TCA GGA GCT GAG TCG CTG TTG TCA CAG GAG AAT GGT AGC CAG    8423
Arg Pro Asp Ala Phe Ile Ser Gly Ala Glu Ser Leu Leu Ser Gln Glu Asn Gly Ser Gln

CAT ATT GCC GGT GCC TGG GTG ACA TCC CTG AGT GAT GTT CGG GTG AAT TTC CCC GGA GC-    8483
His Ile Ala Gly Ala Trp Val Thr Ser Leu Ser Asp Val Arg Val Asn Phe Pro Gly Ala

GAG GAG CCG GTA AAA CGC TGG CAG GGT AAT CTG ACA CCG GTG GTG GTG TAT TTC TGA TGG    8543
Glu Glu Pro Val Lys Arg Trp Gln Gly Asn Leu Thr Pro Val Val Val Tyr Phe xxx

CCCGGCAACGAAAAGTGTTAATACCGATGTGGTGGGTGGTTGGTGTCTGTGTGGCCGCAACCTGTATGGCATGTCTGGT    8622

ACTGACACTGGTGATGTTCTGTGCGGTACTGATGTGGTGGAGAGCATGAAAAACCTTGGGGCAGTAGCTATGCTGCCAT    8701

CGCGGATAACCTCCCTCTACTCTGAGCGTCTATCGTACAACCGGGATACCAACTGGCGAATTTGTCCTGTAGTTCGTTA    8780

TTCAGGACTGCCGTTCTGGTCTGTAACAAATAATGCGCGCCCTTTCTACTCCACTGCATCTGCTGCTTTTTGGCCATTC    8859

GTCGGGCGATCACTTCGTTGATTGTTGACTCGACAAACGCTGTTGAAACCAGCTCACCATACCGGCACATTTCTCCGTA    8938
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FIG. 3—*Continued.*

Immunolabelling was done as described by Simons et al. (22) with the appropriate serum or the K88-specific monoclonal antibody AD11 (24) and goat anti-mouse or goat anti-rabbit antibodies conjugated to 10-nm gold particles.

Isolation and purification of K88 fimbriae. K88ab wild-type or mutant fimbriae were isolated and purified by differential centrifugation as described by Jacobs and De Graaf (3), in both the presence and absence of 2 M urea.

Accession numbers. The nucleotide sequences presented in this report will appear in the EMBL/GenBank/DDBJ nucleotide sequence libraries under the accession numbers Z11699 (ECOFAEEFG) and Z11700 (ECOFAEGHIJ).

RESULTS

Nucleotide sequence analysis of *faeF*, *faeH*, *faeI*, and *faeJ*. The genetic determinant for the biosynthesis of K88ab fimbriae was isolated by molecular cloning of a 9.4-kDa *Hin*dIII-*Cla*I DNA fragment derived from the wild-type plasmid pRI8801. The resultant recombinant plasmid pDB88-8 (Fig. 1) contains all the genetic information required for the production of K88 fimbriae except for a part of the regulatory gene *faeB*. Expression of the cloned K88ab gene cluster is controlled by the P_2 promoter of the cloning vehicle pBR322. The positions of the genes *faeC-faeH* are based on analysis of a DNA segment containing the *Hin*dIII-*Eco*RI (2) DNA fragment (Fig. 1) and its mutant derivatives in *E. coli* minicells (13, 16). The nucleotide sequences of *faeC*, *faeD*, *faeE*, and *faeG* have been determined previously (1, 2, 11, 17).

Analysis of the nucleotide sequence of the region encoding FaeF revealed an open reading frame of 489 nucleotides which started with an ATG codon at nucleotide 4404 (Fig. 2). A putative ribosome binding site was found 8 to 13 bases upstream of the *faeF* initiation codon. Analysis of the encoded amino acid sequence by using the algorithm devel-

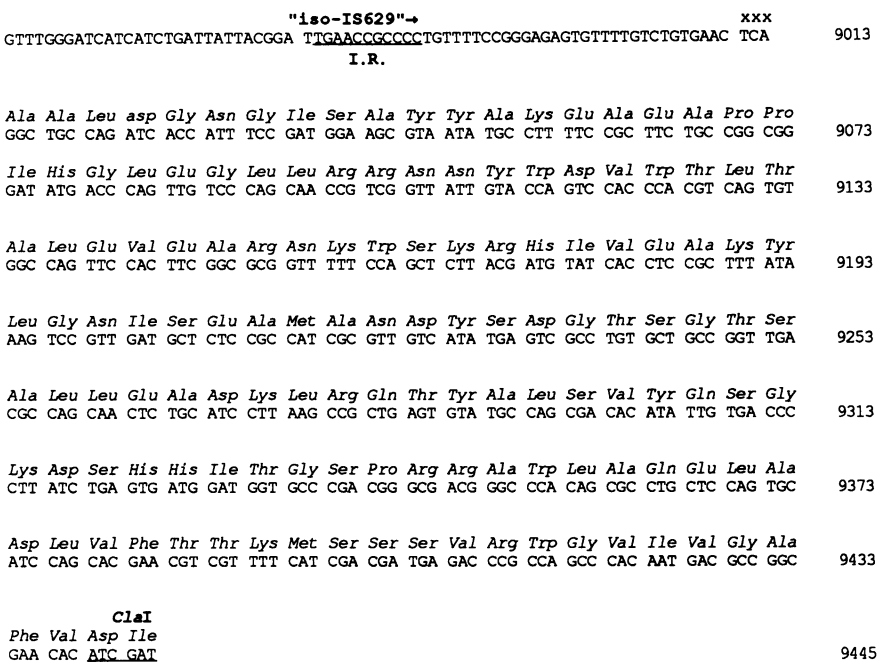

FIG. 3—*Continued.*

oped by Von Heyne (25) predicted the presence of an amino-terminal signal sequence of 22 amino acid residues. The mature protein contains 141 amino acid residues and has a calculated molecular mass of 15,161 Da. The theoretical isoelectric point is 4.79. No promoter or promoterlike structures were detected upstream of *faeF* or in the intercistronic region between *faeF* and *faeG*.

Analysis of the nucleotide sequence downstream of *faeG* confirmed the presence of an open reading frame of 903 nucleotides, designated *faeH*, and separated by 65 bases from *faeG* (Fig. 3).. Three possible initiation codons (H1, H2, and H3) were observed at positions 6051, 6084, and 6159, respectively. However, only initiation at position H3 combines the presence of a putative ribosome binding site 7 bp upstream of the putative start codon with the formation of a polypeptide for which a signal peptidase cleavage site was predicted. The predicted signal sequence consists of 24 amino acid residues, and the mature protein is composed of 241 amino acid residues with a calculated molecular mass of 25,461 Da. The calculated isoelectric point is 4.2, which is close to the experimentally determined isoelectric point of 4.0 (15).

Downstream of *faeH*, another open reading frame was detected which started with an ATG codon at position 6986 and was designated *faeI* (Fig. 3). *faeI* is preceded by a region showing homology to the consensus ribosome binding site. The *faeI* gene product was predicted to be synthesized with a signal sequence of 20 amino acid residues, resulting in a mature protein of 234 amino acid residues, with a calculated molecular mass of 24,804 Da and a pI of 5.1.

Immediately downstream of *faeI*, another open reading frame was detected named *faeJ* (Fig. 3). The putative gene *faeJ* contains 771 nucleotides and is preceded by a putative ribosome binding site. The *faeJ* gene product was predicted to be synthesized with a signal sequence of 26 amino acid residues, resulting in a mature protein of 231 residues, with a calculated molecular mass of 25,093 Da and a pI of 6.6. Analysis of the secondary structure of the *faeJ* mRNA by the method of Jacobsen et al. (5) revealed the presence of a hairpinlike structure close to the ribosome binding site, with a ΔG value of 68.1 kcal/mol (Fig. 4), suggesting that translation of *faeJ* is impaired.

Further downstream, part of an insertion sequence was detected (Fig. 3). Comparison of this sequence with the GenBank data base, using the FASTA program (18), revealed an 86% homology with the insertion sequence IS629, found in *Shigella sonnei* (9). Comparison of the deduced amino acid sequences of FaeF, FaeH, FaeI, and FaeJ with those of other K88-specific proteins revealed similarities between FaeH, FaeI, FaeJ, and the major fimbrial subunit FaeG, in particular, at their amino- and carboxy-terminal ends (Fig. 5). The primary structure of FaeF shows no similarities to other major or minor fimbrial subunits described so far.

Expression of *faeF*, *faeH*, *faeI*, and *faeJ*. Minicells harboring plasmid pDB88-8 or one of its mutant derivatives (Fig. 1) were analyzed for the presence of K88-specific proteins. FaeF, FaeH, and FaeI could be detected in minicells by autoradiography (Fig. 6). FaeI was shown to have an apparent molecular weight slightly higher than that of FaeH (approximately 28,000).

Minicell analysis of a FaeJ mutant did not reveal the existence of an FaeJ polypeptide (data not shown). Possibly, FaeJ is masked by one of the other polypeptides with a similar molecular mass, or alternatively, *faeJ* is translated at a very low level due to the secondary structure of *faeJ* mRNA.

Previous studies on the expression of the major fimbrial subunit FaeG have shown that these subunits are unstable in the absence of the chaperonelike protein FaeE (1). Association of FaeE with FaeG protects the fimbrial subunits against degradation by the periplasmic DegP protease. Because of the similarities observed in the primary structure of FaeG and of FaeH and FaeI, the stability of the latter two proteins was also investigated in an FaeE mutant. It appeared that FaeH and FaeI were not detectable in the

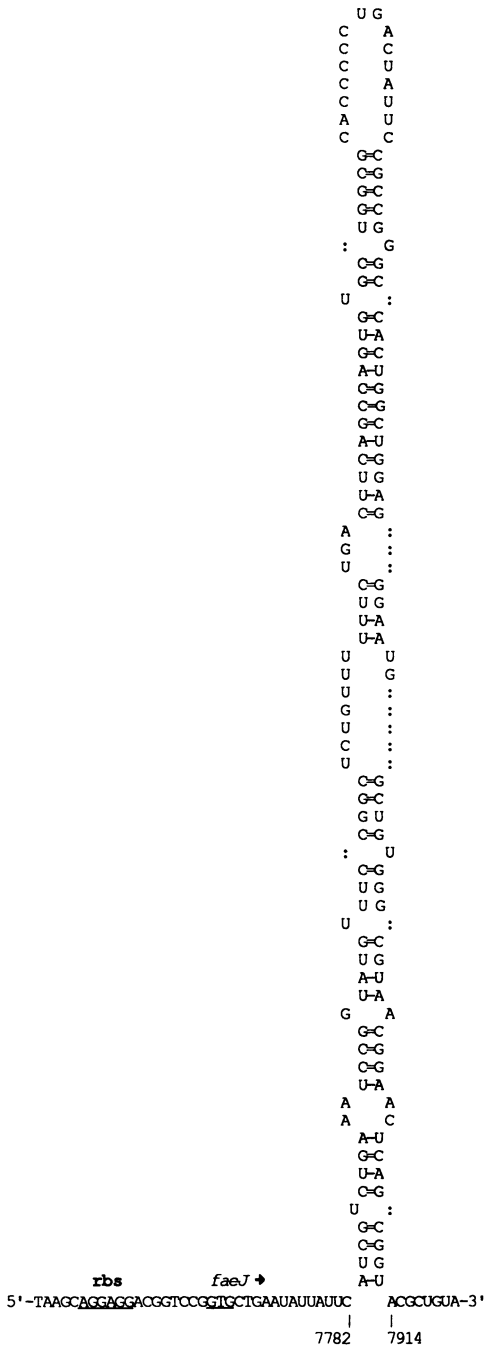

FIG. 4. Predicted secondary structure of the mRNA from the region between nucleotides 7782 and 7914 showing dyad symmetry. The calculated value for ΔG is -68.1 kcal. The 5′ GTG sequence represents the initiation codon of the putative gene *faeJ*.

absence of FaeE (Fig. 6). FaeF, however, was stable in the absence of this chaperonelike protein.

Characterization of FaeF, FaeH, and FaeI mutants. Strains harboring plasmid pDB88-8 or one of its mutant derivatives (Fig. 1) were tested for K88 production. A mutation in *faeF* (pDB88-152) resulted in a 100-fold reduction in the K88 production level as measured in an ELISA. The effect of the *faeF* mutation could be fully restored by introduction of pMK077 into the FaeF mutant. Plasmid pMK077 contains the genes *faeC-faeF* of the K88ac operon (6).

The mutation in *faeH* (pDB88-141) caused a 40% reduction in K88 synthesis. Mutations in *faeI* (pDB88-85) or *faeJ* (pDB88-84) had no significant effect on the biosynthesis of K88 fimbriae.

Investigation of the FaeF and FaeH mutants in the electron microscope demonstrated that FaeF mutant cells carried very few fimbriae and FaeH mutant cells possessed a reduced number of fimbriae compared with cells harboring pDB88-8. No differences were observed in the morphology of fimbriae carried by the various strains (data not shown).

The various mutants were also compared with respect to their adhesive properties. The agglutination of chicken erythrocytes was used as a model system. All mutants agglutinated chicken erythrocytes in proportion to their fimbrial production.

Because treatment of K88 fimbriae with 2 M urea resulted in the removal of the minor fimbrial subunits, the hemagglutinating properties of urea-treated fimbriae were also determined. It appeared that purified K88 fimbriae isolated in the absence or presence of 2 M urea were indistinguishable with respect to their hemagglutinating activity (data not shown). Therefore, the minor fimbrial subunits do not seem to play a role in the agglutination of chicken erythrocytes.

Subcellular localization of FaeF and FaeH. Because both FaeF and FaeH appeared to be essential for K88 biosynthesis, we investigated the subcellular location of these proteins. Previous studies (15) have shown that both proteins are present in the periplasm, but their possible incorporation into K88 fimbriae was not investigated. To test this assumption, we used antisera raised against FaeF-Cro-LacZ and FaeH-Cro-LacZ fusion proteins to detect these proteins in K88 fimbriae. Purified K88 fimbriae were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then immunoblotting with the various antisera (Fig. 7). FaeC antiserum was used as a control (17). Both FaeF and FaeH were detected on the immunoblot.

Treatment of K88 fimbriae with 2 M urea resulted in the removal of the minor fimbrial subunits (Fig. 7). Apparently, both FaeF and FaeH are rather loosely associated with the fimbrial structure, as was previously shown for FaeC (17).

The locations of FaeF and FaeH in K88 fimbriae were further studied by immunoelectron microscopy. Gold particles could be detected along the fimbrial structure, indicating that both proteins are minor components of the K88 fimbriae (Fig. 8). Control experiments using anti-FaeF, anti-FaeG, and anti-FaeH serum with the corresponding mutant phenotypes showed no labelling in immunoelectron microscopy.

Subcellular localization of FaeI appeared to be impossible because of the low specificity of the FaeI antiserum. Several attempts to improve the specificity of the FaeI antiserum have failed.

DISCUSSION

Nucleotide sequence analysis of the promoter-distal end of the K88ab operon elucidated the structures of the genes *faeF* and *faeH* and the hitherto unknown genes *faeI* and *faeJ*. Because of the hairpinlike structure in the 5′ end of *faeJ*, expression of this gene is unlikely. The hairpinlike structure, on the other hand, could function as a stabilizing factor for the K88ab mRNA. Further downstream from *faeJ*, the K88ab operon is flanked by an IS*629*-like insertion sequence.

The results presented in this study show that both FaeF

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FaeG    ---------WMT-G-DFNGSVDIGGSITADDYRQKWEWKVGTGLNGFG-NVLNDLTNGGT-
FaeH    PHADI-----L-DG----GEIQFNGFVT-DDA-PKWTWQISSPDQTWAVDTA-DARTENG-
FaeI    ---------WNTPGEDFSGELKLEGAVTST--RNPWVWKVGQGNESLEVKQSRGVRDGEQ-
FaeJ    HPLTIPPGHWL-EG-MAVGVTELSGTLYVRD--VSWQWQ---PR---AVRMS-SPDAV-QA

FaeG    KLTITVTGNKPILLGRTKEAFATPVSGGVDGI-PQIAFTDYEGASV-KLRNTDGETNKGLA
FaeH    QLVFDLSDKGP-LPF-LEGYLYEVAERGGPGFTPFITFS-SNG----RPFAVKEGSDTSVQ
FaeI    GIPVALPALT-VLLGKT--TLTTP--AGREGLSPGVSYGK--GAEGFSLEWTA----PGMA
FaeJ    GLA-A--GKG----GMV----SE--SRRGQDF--YILGGHTT-----SLTTARSGLQPSVT

FaeG    YFVLPMKNAEGTKVGSVKVNASYAGVFGKGGVTSADGELFSLFADGLRAIFYGGLTTTVS-
FaeH    RF----------R-ASVPVRDPETGNVS--------GQLSFTLNQGMAVSTGKQEEGAST-
FaeI    KVTLPVTGDKNVRAGTFTFRMQAAGVL-----RHM--Q------DGQPV--YTGVYDDLN-
FaeJ    L--L--------QVAPSSPRIAARGELARGQVRY--GEITFTLRHLLA---W-Q--DNITG

FaeG    GAALTSGSAAAARTELFGSLSRN--DILGQIQRVNANITSLVDVAG-SYREDMEY-T----
FaeH    PSGMSLVSGQSVTDVQSGSLPQGLKNRLSALLLMNKGFG-----NGMSAVDNGQVITQGVL
FaeI    ANGLPGESTAMKTSDIPGTL---------QTMFSGEGPSWLQTMTVSGYSGVSHFS-----
FaeJ    GQGWSVVSGE-VTPEAE---KQ-VKRQLWQ---VN-GYEWTPDYAGLTARPDAFISGAESL

FaeG    ---DGTV--VSAAYALGIANGQTIEATFNQAVTTSTQWSAPLNVAITYY
FaeH    --ADGRVMNLAAAYASAVSD---FELRLPAEGTPA-RWQAGLNVTVTVQ
FaeI    ---DASLRQVEGVYGAQIVAG-GGEL--HLNGAMPERWRVSLPVSIEYQ
FaeJ    LSQENGSQHIAGAWVTSLSD---VRVNFPGAEEPVKRWQGNLTPVVVYF
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FIG. 5. Comparison of the primary structures of FaeG, FaeH, FaeI, and FaeJ. The amino acid sequences are given in the single-letter code. Identical amino acid residues are presented by boldface capitals.

and FaeH are minor fimbrial subunits of the K88 fimbriae. Because FaeI and FaeJ share significant homology with FaeH, it is most likely that FaeI and FaeJ are also minor fimbrial subunits, although not essential for K88 biosynthesis. Like FaeG and FaeH, FaeI was not detectable in the absence of FaeE, suggesting that FaeI also requires association with the chaperone to be protected against degradation by the DegP protease.

Fimbriae that were stripped of their minor fimbrial subunits by treatment with 2 M urea still retained their affinity for chicken erythrocytes, indicating that FaeG serves both as a major fimbrial subunit and as an adhesin (4). Furthermore, FaeF and FaeH mutants agglutinated chicken erythrocytes in proportion to their K88 production level. It is not known whether the minor fimbrial subunits, in particular, FaeH and FaeI, are incorporated into the fimbrial structure or only attached along the FaeG polymer. Preliminary experiments have indicated that treatment of K88 fimbriae with 2 M urea results in breakage of the fimbriae into fragments.

A precise function of these minor fimbrial subunits in the biogenesis of the K88 fimbriae cannot be deduced from the experiments. The FaeF mutant exhibited a 100-fold reduction in K88 production level as determined by ELISA, and no fimbrial structures were detectable by electron microscopy. Indications about the role of FaeF in the biosynthesis of K88 fimbriae cannot be derived from its primary structure. FaeF, however, is required at some step in the initiation and/or elongation of the K88 fimbriae. A functionally comparable protein, FanF, has been identified in the K99 system (22). Mutations in FanF result in the production of fewer and apparently much shorter K99 fimbriae.

The FaeH mutant produced fewer fimbriae than cells

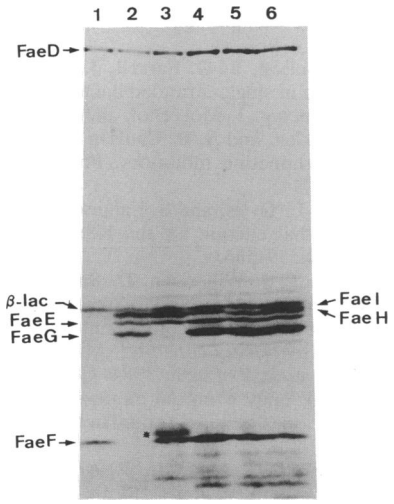

FIG. 6. Autoradiograph of minicells harboring pDB88-8 and its mutant derivatives. The positions of the major and minor fimbrial subunits and of β-lactamase (β-lac) are indicated. Lanes: 1, FaeE mutant; 2, FaeF mutant; 3, FaeG mutant; 4, FaeH mutant; 5, FaeI mutant; and 6, minicells harboring pDB88-8 (control). The asterisk indicates the position of an FaeG truncate.

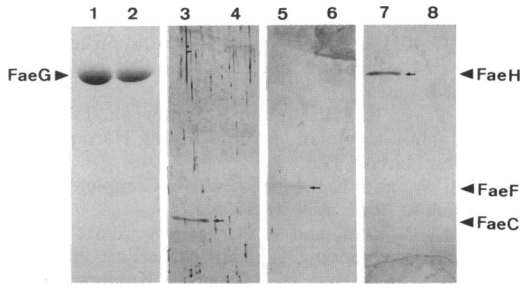

FIG. 7. Detection of the minor components FaeC, FaeF, and FaeH in fimbriae isolated in the presence (lanes 2, 4, 6, and 8) or absence (lanes 1, 3, 5, and 7) of 2 M urea. Lanes 1 and 2, SDS-PAGE stained with Coomassie blue; lanes 3 to 8, immunoblots developed with specific antisera against FaeC (lanes 3 and 4), FaeF (lanes 5 and 6), and FaeH (lanes 7 and 8).

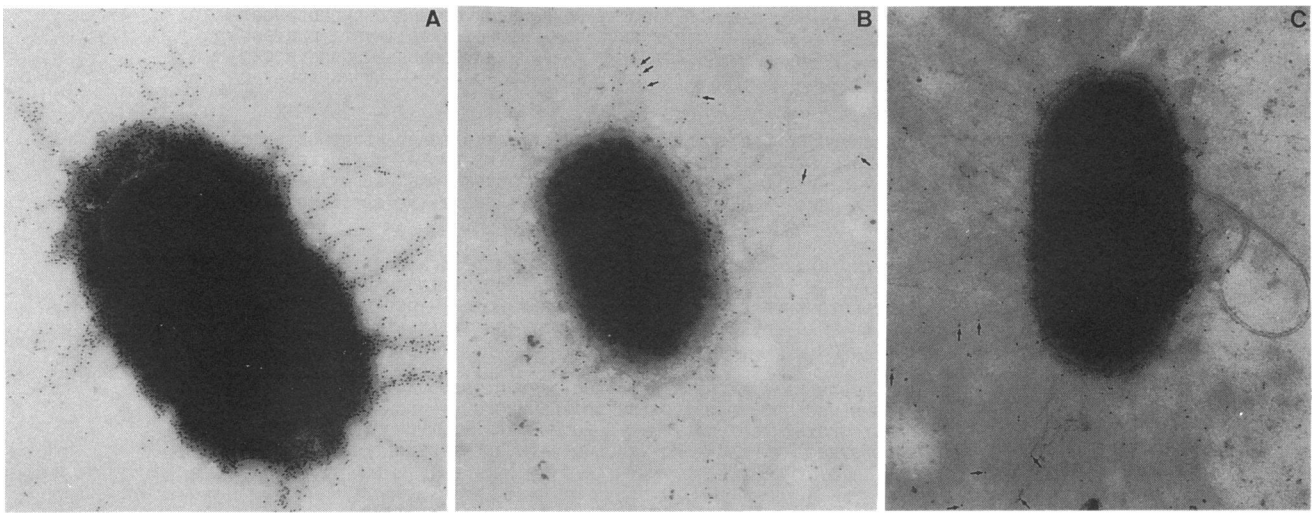

FIG. 8. Immunoelectron microscopy. Cells harboring plasmid pDB88-8 were cultured and treated as described in Materials and Methods. (A) K88ab fimbriae incubated with monoclonal antiserum (AD11); (B) K88ab fimbriae incubated with antiserum against FaeH; (C) K88ab fimbriae incubated with antiserum against FaeF. Arrows indicate the positions of some fimbria-associated gold particles.

harboring an intact K88 operon. FaeH$^-$ fimbriae, however, were functionally and morphologically indistinguishable from wild-type fimbriae.

REFERENCES

1. **Bakker, D., C. E. M. Vader, B. Roosendaal, F. R. Mooi, B. Oudega, and F. K. De Graaf.** 1991. Structure and function of periplasmic chaperone-like proteins involved in the biosynthesis of K88 and K99 fimbriae in enterotoxigenic *Escherichia coli*. Mol. Microbiol. **5**:875–886.
2. **Gaastra, W., F. R. Mooi, A. R. Stuitje, and F. K. De Graaf.** 1981. The nucleotide sequence of the gene encoding the K88ab protein subunit of porcine enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **12**:41–46.
3. **Jacobs, A. A. C., and F. K. De Graaf.** 1985. Production of K88, K99 and F41 fibrillae in relation to growth phase, and a rapid procedure for adhesin purification. FEMS Microbiol. Lett. **26**:15–19.
4. **Jacobs, A. A. C., B. Roosendaal, J. F. L. Van Breemen, and F. K. De Graaf.** 1987. Role of phenylalanine-150 in the receptor binding domain of the K88 fibrillar subunit. J. Bacteriol. **169**: 4907–4911.
5. **Jacobsen, A. B., L. Good, J. Simonetti, and M. Zuker.** 1984. Some simple computational methods to improve folding of long mRNAs. Nucleic Acids Res. **12**:45–52.
6. **Kehoe, M., R. Sellwood, P. Shipley, and G. Dougan.** 1981. Genetic analysis of K88-mediated adhesion of enterotoxigenic *Escherichia coli*. Nature (London) **291**:122–126.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
8. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Matsutani, S., and E. Ohtsubo.** 1990. Complete sequence of IS629. Nucleic Acids Res. **18**:1899.
10. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
11. **Mooi, F. R., I. Claassen, D. Bakker, H. Kuipers, and F. K. De Graaf.** 1986. Regulation and structure of an *Escherichia coli* gene coding for an outer membrane protein involved in export of K88ab fimbrial subunits. Nucleic Acids Res. **14**:2443–2457.
12. **Mooi, F. R., F. K. De Graaf, and J. D. A. Van Embden.** 1979. Cloning, mapping and expression of the genetic determinant that encodes for the K88ab antigen. Nucleic Acids Res. **6**:849–865.
13. **Mooi, F. R., N. Harms, D. Bakker, and F. K. De Graaf.** 1981. Organization and expression of genes involved in the production of the K88ab antigen. Infect. Immun. **32**:1155–1163.
14. **Mooi, F. R., M. Van Buuren, G. Koopman, B. Roosendaal, and F. K. De Graaf.** 1984. A K88ab gene of *Escherichia coli* encodes a fimbria-like protein distinct from the K88ab fimbrial adhesin. J. Bacteriol. **159**:482–487.
15. **Mooi, F. R., A. Wijffjes, and F. K. De Graaf.** 1983. Identification and characterization of precursors in the biosynthesis of the K88ab fimbriae of *Escherichia coli*. J. Bacteriol. **154**:41–49.
16. **Mooi, F. R., C. Wouters, A. Wijffjes, and F. K. De Graaf.** 1982. Construction and characterization of mutants impaired in the biosynthesis of the K88ab antigen. J. Bacteriol. **150**:512–521.
17. **Oudega, B., M. De Graaf, L. De Boer, D. Bakker, C. E. M. Vader, F. R. Mooi, and F. K. De Graaf.** 1989. Detection and identification of FaeC as a minor component of K88 fibrillae of *Escherichia coli*. Mol. Microbiol. **3**:645–652.
18. **Pearson, W. R., and D. J. Lipman.** 1983. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2485.
19. **Sanger, F., R. Coulson, B. G. Barrell, J. H. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
20. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
21. **Shipley, P. L., C. L. Gyles, and S. Falkow.** 1978. Characterization of plasmids that encode for the K88 colonization factor. Infect. Immun. **20**:559–566.
22. **Simons, B. L., P. T. J. Willemsen, D. Bakker, B. Roosendaal, F. K. De Graaf, and B. Oudega.** 1990. Structure, localization and function of FanF, a minor component of K99 fibrillae of enterotoxigenic *Escherichia coli*. Mol. Microbiol. **4**:2041–2050.
23. **Stanley, K. K., and J. P. Luzio.** 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. EMBO J. **3**:1429–1434.
24. **Van Zijderveld, F. G., J. Anakotta, R. A. M. Brouwers, A. M. Van Zijderveld, D. Bakker, and F. K. De Graaf.** 1990. Epitope analysis of the F4 (K88) fimbrial antigen complex of enterotoxigenic *Escherichia coli* by using monoclonal antibodies. Infect. Immun. **58**:1870–1878.
25. **Von Heyne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14**:4683–4690.