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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FaoE → CCG GAA GCC GCA AAA GCT GAG AAA GCA GAT ACC GCA GAG CAG AAA TAA CCGCCCTCCGGAATA 4384 Pro Glu Ala Ala Lys Ala Glu Lys Ala Asp Thr Ala Glu Gln Lys xxx CGGAACAGAGGGGTAATAA ATG AAG AAA ACA ATG ATG GCA GCC GCC CTG GTT CTG AGT GCG CTC 4448 Met Lys Lys Thr Met Met Ala Ala Ala Leu Val Leu Ser Ala Leu rbs TaoT -Nsp BII AGT ATT CAG TCA GCA CTG G<u>CC GCT G</u>AA TAC AGT GAG AAA ACG CAG TAC CTG GGT GTG GTG Ser Ile Gln Ser Ala Leu Ala Ala Glu Tyr Ser Glu Lys Thr Gln Tyr Leu Gly Val Val 4508 AAC GGT CAG GTG GTG GGT AAC AGT GTG GTG AAG GTG ACC CGT ACA CCG ACA GAC CCG GTG Asn Gly Gln Val Val Gly Asn Ser Val Val Lys Val Thr Arg Thr Pro Thr Asp Pro Val 4568 CTG TAC CGC TCC GGC AGT AAC AGC CCG TTA CCT GCA GAA CTG ATA ATC CGG CAT GCA GAA 4628 Leu Tyr Arg Ser Gly Ser Asn Ser Pro Leu Pro Ala Glu Leu Ile Ile Arg His Ala Glu AGC CGC CCG GCT TCC GGC GGC CTG GCA AAC ATC ACG GTG AAA GAG GCG CTG CCG GAT AAC Ser Arg Pro Ala Ser Gly Gly Leu Ala Asn Ile Thr Val Lys Glu Ala Leu Pro Asp Asn 4688 GGG GAA GCC CGC ATC ACT CTG AAG ACG TCC CTG ATG GTT GAC GGA AAG AGA GTG GCA CTC 4748 Gly Glu Ala Arg Ile Thr Leu Lys Thr Ser Leu Met Val Asp Gly Lys Arg Val Ala Leu Bsu 36I AGT GCC AGG CAG CAG GGT GAG GAT GTG GTG ATT ACC GTG CCT GAG GCA CAG CAG CAG ATT 4808 Ser Ala Arg Gln Gln Gly Glu Asp Val Val Ile Thr Val Pro Glu Ala Gln Gln Gln Ile GAG TTA AGA ACA GAT GCA CCG GCA GAG CTG GAG GTG CCG GTC AGC TAC CGG GGA AAC CTG 4868 Glu Leu Arg Thr Asp Ala Pro Ala Glu Leu Glu Val Pro Val Ser Tyr Arg Gly Asn Leu CAG ATA GCG CTG CAG GTG GAG GAC TGA GGATTAATCTCCTTAGTGATGCAAAACATCCGTGACTCCGGAA 4938 Gln Ile Ala Leu Gln Val Glu Asp xxx ${\tt CGGTCATATTGGTAAAGGGACTTGCCGTTTTTTTAAACGGGAATAACGCAAAGCTGTTCTGGGTTAAACACAGTGTTT$ 5017 AATGAAATGCGGTTATTTAAACGGAGCCGCAGGGATAGTTTTACGGTAATTCCGGAAAAATAAGGGTTACCGATTTCAG 5096 TTTATTATTTGTGGAATATCAAGGGGTTTATTTT ATG AAA AAG ACT CTG ATT GCA CTG GCA ATT GCT 5163 rbs Met Lys Lys Thr Leu Ile Ala Leu Ala Ile Ala TagG -GCA TCT GCT GCA TCT GCT ATG GCA CAT GCC TGG ATG ACT GGT GAT TTC AAT GGT TCG GTC 5223 Ala Ser Ala Ala Ser Gly Met Ala His Ala Trp Met Thr Gly Asp Phe Asn Gly Ser Val EcoR GAT ATC GGT GGT AGT ATC ACT GCA GAT GAT TAT CGT CAG AAA TGG GAA TGG AAA GTT GGT 5283 Asp Ile Gly Gly Ser Ile Thr Ala Asp Asp Tyr Arg Gln Lys Trp Glu Trp Lys Val Gly

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 (\uparrow) 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 sup-

plemented with chloramphenicol (40 μ g/ml), ampicillin (200 μ g/ml), and/or kanamycin (50 μ g/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 *NspBII-EcoRV* fragment containing nucleotides 4470 to 5226 in the *SmaI* 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,

TaeG-

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ACT Thr	CAG Gln	TGG Trp	AGC Ser	GCT Ala	CCG Pro	CTG Leu	AAC Asn	GTA Val	GCA Ala	ATT Ile	ACT Thr	TAT Tyr	TAC Tyr	TAA XXX	GTTO	STCG1	GATO	GAGCI	G	6005
CCAR	ለጥጥጥፖ	\ ጥጥል ነ	TGA	PACGI	TCTO	ATA	CAG	Bst	XI/2	Kbal	(H1 T G1) 'G GA	C GC	т ст	יד די	יד איז	יד הי	'G AG	G	6074
COL											Va	il As	sp Al	la Le	eu Pl	ne Il	Le Va	al An	rg	0071
			(H2)																
TTT Phe	ATA Ile	CTG Leu	ATG Met	CTG Leu	GTT Val	ACT Thr	TTA Leu	AAT Asn	TTA Leu	ATA Ile	ATA Ile	GCG Ala	ATT Ile	TGT Cys	TTT Phe	TAT Tyr	TTG Leu	TAT Tyr	TGT Cys	6134
				rbs				(H3)												
CCT	GAT	AAA	ACA	GAG	AGA	ATA	AAG	ATG	AAA	ATA	ACG	CAT	CAT	TAT	AAA	TCC	CTT	CTT	TCA	6194
Pro	Asp	Lys	Thr	GIU	Arg	шe	Lys	Met Fa	Lys weHi⊸))	Inr	HIS	HIS	Tyr	Lys	Ser	Leu	Leu	Ser	
GCC	ATT	ATT	TCG	GTG	GCC	CTT	TTT	TAT	TCG	GCA	GCG	CCA	CAT	GCA	GAT	ATT	CTT	GAT	GGT	6254
Ala	Ile	Ile	Ser	Val	Ala	Leu	Phe	Tyr	Ser	Ala	Ala	Pro	His	Ala	Asp	Ile	Leu	Asp	Gly	
																Reti	ат			
GGC	GAA	ATT	CAG	TTŤ	AAT	GGC	TTT	GTC	ACT	GAT	GAT	GCC	ccc	AAA	TGG	ACC	TGG	CAG	ATT	6314
Gly	Glu	Ile	Gln	Phe	Asn	Gly	Phe	Val	Thr	Asp	Asp	Ala	Pro	Lys	Trp	Thr	Trp	Gln.	Ile	
AGT	TCA	CCG	GAC	CAG	АСТ	TGG	GCT	GTG	GAT	АСТ	GCC	GAT	GCA	CGT	ACA	GAG	AAC	GGG	CAA	6374
Ser	Ser	Pro	Asp	Gln	Thr	Trp	Ala	Val	Asp	Thr	Ala	Asp	Ala	Arg	Thr	Glu	Asn	Gly	Gln	
Ecc0109I																				
CTG	GTT Val	TTT Phe	GAT	TTG	AGT	GAC	AAA Lvs	<u>GGG</u> Glv	CCT Pro	CTG	CCT	TTT Phe	CTT	GAG Glu	GGG Glv	TAT	TTG Leu	TAT Tvr	GAA Glu	6434
200	·ui			200			-1-						200		1	-1-		-1-		
GTG	GCC	GAG	CGT	GGC	GGT	ccc	GGA	TTC	ACG	ccc	TTT	ATT	ACT	TTC	AGC	AGT	AAC	GGA	CGG	6494
Val	Ala	Glu	Arg	GIY	GIY	Pro	GIY	Phe	Thr	Pro	Phe	Ile	Thr	Phe	Ser	Ser	Asn	GIY	Arg	
ССТ	TŤC	GCC	GTA	AAG	GAA	GGC	AGT	GÁC	ACT	TCA	GTG	CAA	CGT	TTT	CGC	GCC	TCT	GTC	CCG	6554
Pro	Phe	Ala	Val	Lys	Glu	Gly	Ser	Asp	Thr	Ser	Val	Gln	Arg	Phe	Arg	Ala	Ser	Val	Pro	
	~~~	~ ~	~~~	~~~	100	~~~		0.000		~~~	~~~	000	BOB		100	0.000		~		6614
Val	Arg	Asp	Pro	Glu	Thr	Gly	Asn	Val	Ser	Gly	Gln	Leu	Ser	Phe	Thr	Leu	Asn	Gln	Gly	0014
ATG Met	GCG	GTC Val	AGT Ser	ACA Thr	GGT	AAA	CAG Gln	GAA Glu	GAG Glu	GGC Glv	GCC Ala	TCC Ser	ACG Thr	CCT	TCT	GGT	ATG Met	TCA Ser	CTG Leu	6674
		vur	001		<b>0</b> 17	270			010	017		001		110	001	011		001	Deu	
GTC	AGT	GGA	CAA	AGT	GTG	ACA	GAT	GTT	CAG	TCA	GGC	AGT	CTT	CCG	CAG	GGG	CTG	aag	AAC	6734
Val	Ser	Gly	Gln	Ser	Val	Thr	Asp	Val	Gln	Ser	Gly	Ser	Leu	Pro	Gln	Gly	Leu	Lys	Asn	
CGT	CTG	тст	GCC	TTA	TTG	CTG	ATG	ААТ	AAG	GGG	TTC	GGT	ААТ	GGC	ATG	AGT	GCG	GTG	GAT	6794
Arg	Leu	Ser	Ala	Leu	Leu	Leu	Met	Asn	Lys	Gly	Phe	Gly	Asn	Gly	Met	Ser	Ala	Val	Asp	
AAC Asn	GGA Gly	Gln	Val	ATC Ile	ACT Thr	CAG Gln	GGG Gly	GTA Val	CTG Leu	GCT Ala	GAC	GGT Gly	CGT Arg	GTG Val	ATG Met	AAT Asn	CTG Leu	GCT Ala	GCG Ala	6854
	_						_				-	-	-							
GCA	TAT	GCC	TCT	GCG	GTG	TCG	GAT	TTT	GAA	CTG	CGG	TTG	CCG	GCG	GAA	GGC	ACA	CCG	GCC	6914
ALA	Tyr	Ata	Ser	A⊥a	vai	Ser	Asp	Pne	GIU	Leu	Arg	Leu	Pro	Ala	GIU	GIY	Inr	Pro	AIA	

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 IS629-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 ( $\uparrow$ ), and the inverted repeat (I.R.) of IS629 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 *SmaI* 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 *Hinc*II fragment encompassing nucleotides 7218 to 8356 (see Fig. 3) into the *SmaI* 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 *Bsu36I*, 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-

6979

Arg	Trp	Gln	Ala	Gly	Leu	Asn	Val	Thr	Val	Thr	Val	Gln	XXX					rb	s	
ATAG	AA I	ATG A Met 1 <b>Fae</b>	AAG A Lys I I →	NAG ( Lys ∖	GTG # /al T	ACG I Thr I	TG T æu P	TT C he L	TG I eu P	TT G he V	TT G al V	TC A al S	GC C er I	TC C eu I	TG C eu P	CC T ro S	B CC A er T	stXI <u>CT G</u> hr V	<u>TA</u> al	7039
<u>CTG</u> Leu	BstN GCC Ala	NI TGG Trp t	AAC Asn	ACG Thr	CCG Pro	GGA Gly	GAA Glu	GAC Asp	TTC Phe	AGC Ser	GGA Gly	GAG Glu	CTT Leu	AAG Lys	CTG Leu	GAA Glu	GGG Gly	GCG Ala	GTG Val	7099
ACC Thr	AGC Ser	ACC Thr	CGT Arg	AAT Asn	CCG Pro	TGG Trp	GTG Val	TGG Trp	AAA Lys	GTC Val	GGA Gly	CAG Gln	GGA Gly	AAT Asn	GAA Glu	AGT Ser	CTG Leu	GAG Glu	GTT Val	7159
AAG Lys	C <b>A</b> G Gln	AGC Ser	CGT Arg	GGT Gly	GTT Val	CGT Arg	GAC Asp	GGT Gly	GAG Glu	CAG Gln	Ec GGA Gly	co RI <u>ATT</u> Ile	<u>C</u> CG Pro	GTT Val	GCA Ala	CTG Leu	CCG Pro	<i>Hin</i> GC <u>G</u> Ala	cII TTG Leu	7219
<u>AC</u> C Thr	GTT Val	TTA Leu	CTG Leu	GGA Gly	AAA Lys	ACC Thr	ACC Thr	CTG Leu	ACC Thr	ACA Thr	CCG Pro	GCA Ala	GGA Gly	CGT Arg	GAG Glu	GGG Gly	CTT Leu	TCC Ser	CCC Pro	7279
GGG Gly	GTC Val	AGT Ser	TAC Tyr	GGA Gly	AAG Lys	GGG Gly	GCT Ala	GAG Glu	GGT Gly	TTT Phe	TCA Ser	CTT Leu	GAA Glu	TGG Trp	ACA Thr	GCG Ala	CCC Pro	GGC Gly	ATG Met	7339
GCG Ala	AAA Lys	GTG Val	' ACG Thr	CTG Leu	CCT Pro	GTG Val	ACC Thr	GGC Gly	GAT Asp	AAA Lys	AAT Asn	GTT Val	CGT Arg	GCG Ala	GGG Gly	ACA Thr	TTC Phe	ACC Thr	TTC Phe	7399
AGG Arg	ATG Met	G CAG	GCG Ala	GCC Ala	GGG Gly	GTG Val	TTG Leu	CGT Arg	CAT His	ATG Met	C <b>A</b> G Gln	GAC Asp	GGA Gly	C <b>AA</b> Gln	CCG Pro	GTG Val	TAT Tyr	ACC Thr	GGC Gly	7459
GTA Val	ТАТ Туг	GAC	GAC Asp	CTG Leu	AAT Asn	GCG Ala	AAT Asn	GGG Gly	CTG Leu	CCG Pro	GGT GLY	GAA Glu	AGC Ser	ACA Thr	GCC Ala	ATG Met	AAG Lys	ACT Thr	TCT Ser	7519
GAT Asp	ATI Ile	CCG Pro	GGG Gly	ACT Thr	CTG Leu	C <b>A</b> G Gln	ACG Thr	ATG Met	TTC Phe	AGT Ser	GGT Gly	G <b>AA</b> Glu	GGT Gly	CCG Pro	TCC Ser	TGG Trp	CTG Leu	C <b>A</b> G Gln	ACA Thr	7579
ATG Met	ACA Thr	A GTC Val	AGT Ser	GGT Gly	TAT Tyr	TCG Ser	GGA Gly	GTG Val	AGT Ser	CAT His	TTC Phe	AGT Ser	GAT Asp	GCC Ala	TCC Ser	CTG Leu	CGT Arg	C <b>A</b> G Gln	GTT Val	7639
G <b>AA</b> Glu	GG1 Gly	r GTG / Val	TAC Tyr	GGC Gly	GCA Ala	C <b>A</b> G Gln	ATT Ile	GTG Val	GCA Ala	GGC Gly	GGT Gly	GGT Gly	GAA Glu	TTA Leu	CAT His	CTG Leu	AAC Asn	GGC Gly	GCG Ala	7699
ATG Met	CCG Pro	G GAA	CGC Arg	TGG Trp	CGG Arg	GTG Val	TCA Ser	CTG Leu	CCG Pro	GTA Val	AGT Ser	ATT Ile	GAG Glu	ТАС 1 Тут	CAG Gln	TAA XXX	GCAC	GAGO ZDS	ACGG	7762
тсс	G GI Va Fa	IG CI 11 Le 20J	GAA ⊔As	T AT n Il	TAT eIl	T CA e Hi	T CG s Ar	T CT g Le	G AA u Ly	A TC s Se	C GG r Gl	TAT YMe	G TT t Ph	T CC e Pr	G GC 0 Al	T CTO a Leo	G TT. J Phe	r TTI e Phe	CTG E Leu	7823
			Pat																	

CGC TGG CAG GCA GGG CTG AAT GTG ACA GTC ACG GTA CAG TGA TGGTCGCAGGGATATAAGGAGAA

ACT TCA GC<u>C AGT GTG CTG G</u>CG CAC CCC CTG ACT ATT CCG CCG GGC CAC TGG CTG GAG GGA 7883 Thr Ser Ala Ser Val Leu Ala His Pro Leu Thr Ile Pro Pro Gly His Trp Leu Glu Gly

FIG. 3—Continued.

3'. The resulting plasmid was named pDB88-141. The deletion mutation in *faeI* was constructed by digesting pDB88-8 with *BstXI* and religating with T4 DNA ligase, which resulted in removal of the *BstXI* fragment between nucleotides 7039 and 7838 (see Fig. 3). A frameshift mutation in *faeJ* was constructed by insertion of a *ClaI* linker into the unique *HpaI-HincII* 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  $\mu$ Ci/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	C <b>A</b> G	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	
CGC Arg	CAG Gln	TTA Leu	TGG Trp	CAG Gln	GTG Val	AAC Asn	GGC Gly	TAT Tyr	G <b>AA</b> Glu	TGG Trp	ACC Thr	CCG Pro	Gac Asp	TAT Tyr	GCC Ala	F GG <u>G</u> Gly	<i>linc</i> I <u>TTA</u> Leu	I <u>AC</u> C Thr	GCG Ala	8363
CGT	CCT	GAT	GCG	TTT	ATT	TCA	GGA	GCT	GAG	TCG	CTG	TTG	TCA	C <b>A</b> G	GAG	AAT	GGT	AGC	C <b>A</b> G	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 Glu	GAG Glu	CCG Pro	GTA Val	AAA Lys	CGC Arg	TGG Trp	CAG Gln	GGT Gly	AAT Asn	CTG Leu	ACA Thr	CCG Pro	GTG Val	GTG Val	GTG Val	TAT Tyr	TTC Phe	TGA XXX	TGG	8543
ccca	GCA	ACGA	AAAG:	(GTT)	<b>A</b> TAC	CCGA	IGTG	GTGGG	GTGG	rtgg:	IGTC:	IGTG	rggco	CGCA	ACCTO	GTATO	GCA.	IGTC	IGGT	8622
ACTO	GACAC	CTGG	IGATO	GTTC	IGTGC	CGGT	ACTG/	ATGTO	GGTG	GAGA	GCAT	GAAA	AACC	ITGG	GCA	GTAG	CTATO	GCTG	CCAT	8701
CGCC	GAT	ACC	ICCC:	ICTAG	стсто	GAGCO	GTCT	ATCG:	[ACA)	ACCG	GAT	ACCA	ACTG	GCGA	ATTT	STCC	IGTA	GTTC	GTTA	8780
TTCA	GGAC	CTGCO	CGTTO	CTGG	ICTGI	[AAC]	AAT/	ATG	CGCG	CCT	TCT	ACTCO	CACTO	GCATO	CTGC	IGCT	TTT	GCC.	ATTC	8859
GTCO	GGCC	GATC	ACTTO	CGTTO	GATTO	GTTG/	ACTCO	GACAA	AACGO	стбті <b>3—(</b>	igaan Cont	ACCAO inue	эстс <i>і</i> d.	ACCA	FACCO	GCA	CATT	ICTC(	CGTA	8938

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 HindIII-ClaI 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 *HindIII-EcoRI* (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-

GTTI	"iso-IS629"→ XXX GTTTGGGATCATCTGATTATTACGGA T <u>TGAACCGCCCC</u> TGTTTTCCGGGAGAGTGTTTTGTCTGTGAAC TCA I.R.											9013								
Ala	Ala	<i>Leu</i>	<i>asp</i>	Gly	Asn	Gly	Ile	Ser	Ala	<i>Tyr</i>	<i>Tyr</i>	Ala	<i>Lys</i>	<i>Glu</i>	Ala	<i>Glu</i>	Ala	<i>Pro</i>	Pro	9073
GGC	TGC	CAG	ATC	ACC	ATT	TCC	GAT	GGA	AGC	GTA	ATA	TGC	CTT	TTC	CGC	TTC	TGC	CGG	CGG	
Ile	<i>His</i>	Gly	<i>Leu</i>	<i>Glu</i>	<i>Gly</i>	<i>Leu</i>	<i>Leu</i>	Arg	Arg	<b>As</b> n	<i>Asn</i>	<i>Tyr</i>	<i>Trp</i>	Asp	Val	<i>Trp</i>	<i>Thr</i>	Leu	<i>Thr</i>	9133
GAT	ATG	ACC	CAG	TTG	TCC	CAG	CAA	CCG	TCG	GTT	ATT	GTA	CCA	GTC	CAC	CCA	CGT	CAG	TGT	
Ala	<i>Leu</i>	<i>Glu</i>	<i>Val</i>	<i>Glu</i>	Ala	Arg	<i>Asn</i>	<i>Lys</i>	Trp	Ser	<i>Lys</i>	Arg	<i>His</i>	Ile	Val	Glu	Ala	<i>Lys</i>	<i>Tyr</i>	9193
GGC	CAG	TTC	CAC	TTC	GGC	GCG	GTT	TTT	CCA	GCT	CTT	ACG	ATG	TAT	CAC	CTC	CGC	TTT	ATA	
<i>Leu</i>	Gly	A <i>sn</i>	Ile	Ser	Glu	Ala	<i>M</i> et	Ala	Asn	Asp	<i>Tyr</i>	<i>Ser</i>	Asp	Gly	<i>Thr</i>	Ser	Gly	Thr	Ser	9253
AAG	TCC	GTT	GAT	GCT	CTC	CGC	CAT	CGC	GTT	GTC	ATA	TGA	GTC	GCC	TGT	GCT	GCC	GGT	TGA	
Ala	<i>Leu</i>	<i>Leu</i>	<i>Glu</i>	Ala	Asp	<i>Lys</i>	Leu	Arg	Gln	<i>Thr</i>	<i>Tyr</i>	Ala	<i>Leu</i>	Ser	Val	<i>Tyr</i>	Gln	<i>Ser</i>	Gly	9313
CGC	CAG	CAA	CTC	TGC	ATC	CTT	AAG	CCG	CTG	AGT	GTA	TGC	CAG	CGA	CAC	ATA	TTG	TGA	CCC	
<i>Lys</i>	Asp	Ser	<i>Hís</i>	<i>His</i>	Ile	<i>Thr</i>	Gly	<i>Ser</i>	<i>Pro</i>	Arg	Arg	Ala	<i>Trp</i>	<i>Leu</i>	Ala	Gln	Glu	<i>Leu</i>	Ala	9373
CTT	ATC	TGA	GTG	ATG	GAT	GGT	GCC	CGA	CGG	GCG	ACG	GGC	CCA	CAG	CGC	CTG	CTC	CAG	TGC	
A <i>sp</i>	<i>Leu</i>	Val	Phe	<i>Thr</i>	Thr	<i>Lys</i>	<i>Met</i>	Ser	Ser	Ser	Val	Arg	<i>Trp</i>	Gly	Val	Ile	Val	Gly	Ala	9433
ATC	CAG	CAC	GAA	CGT	CGT	TTT	CAT	CGA	CGA	TGA	GAC	CCG	CCA	GCC	CAC	AAT	GAC	GCC	GGC	
Phe GAA	Val CAC	C Asp <u>ATC</u>	<b>laI</b> Ile GAT					FIG	<b>J.</b> 3	Ce	ontir	nued								9445

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 sclose 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 *fael*, 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  $\Delta G$  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

CCCCCAC GUACUGGCUGACUSAG A G U C≔G UG U-A U-A UUUGUCU U G :::: C=G G=C G U C=G C=G UG UG G=C UG A-U U-A G=C C=G C=G U−A A−U G=C U−A C=G G≕C C≕G ŪG rbs faeJ 🕈 A-U 5"-TAAGCAGGAGGACGGTCCGGTGCTGAAUAUUAUUC ACGCUGUA-3 7914 7782

υG

FIG. 4. Predicted secondary structure of the mRNA from the region between nucleotides 7782 and 7914 showing dyad symmetry. The calculated value for  $\Delta G$  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 IS629-like insertion sequence.

The results presented in this study show that both FaeF

FaeG	WMT-G-DFNGSVDIGGSITADDYRQKWEWKVGTGLNGFG-NVLNDLTNGGT-
FaeH	PHADIL-DGGEIQFNGFVT-DDA-PKWTWQISSPDQTWAVDTA-DARTENG-
Fael	WNTPGEDFSGELKLEGAVTSTRNPWVWKVGQGNESLEVKQSRGVRDGEQ-
FaeJ	HPLTIPPGHWL-EG-MAVGVTELSGTLYVRDVSWQWQPRAVRMS-SPDAV-QA
FaeG	KLTITVTGNKPILLGRTKEAFATPVSGGVDGI-PQIAFTDYEGASV-KLRNTDGETNKGLA
FaeH	QLVFDLSDKGP-LPF-LEGYLYEVAERGGPGFTPFITFS-SNGRPFAVKEGSDT <b>SV</b> Q
Fael	GIPVALPALT-VLLGKTTLTTPAGREGLSPGVSYGKGAEGFSLEWTAPGMA
FaeJ	GLA-AGKGGMVSESRRGQDFYILGGHTTSLTTARSGLQPSVT
FaeG	YFVLPMKNAEGTK <b>UGSVKVNA</b> SY <b>AGV</b> FGKGGVTSADGELFSLFADGLRATFYGGLTTTVS-
FaeH	FR-ASVPVRDPETGNVSGOLSFTLNOGMAVSTGKOEEGAST-
Fael	KYTLPVTGDKNVRAGTFTFRMOAGVLRHM-ODGOPV-YTGVYDDLN-
FaeJ	LLQVAPSSPRIAARGELARGQVRYGEITFTLRHLLAW-QDNITG
FaeG	GAALTSGRAAAARTELFGRISENDILGOIORVNANITSLUDVAG-SVEEDMEV-T
FaeH	PSGMSI USGOSUTOVOSGSLPOGLENRLSALLIMNKGFGNGMSAVDNGOVI TOGVL
Fael	ANGLEGESTAMKTSDIEGTLOTMESGEGESWLOTMTVSGYSGVSHES
FaeJ	GQGWSVVSGE-VTPEAEKQ-VKRQLWQVN-GYEWTPDYAGLTARPDAFISGAESL
FaeG	
FaeH	
Fael	
FaeJ	LSOENGSOH I AGAWVTSLSDVRVNFPGAEEPVKRWOGNLTPVVVYF

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 eryth-



rocytes 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  $\beta$ -lactamase ( $\beta$ -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.

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