Adhesive Factor/Rabbit 2, a New Fimbrial Adhesin and a Virulence Factor from *Escherichia coli* O103, a Serogroup Enteropathogenic for Rabbits

FRANK FIEDERLING, MICHELE BOURY, CLAUDE PETIT, AND ALAIN MILON*

Unité Associée de Microbiologie Moléculaire, Institut National de la Recherche Agronomique/Ecole Nationale Vétérinaire, Ecole Nationale Vétérinaire, F-31076 Toulouse Cedex 3, France

Enteropathogenic *Escherichia coli*-like *E. coli* strains belonging to serovar O103:K⁻:H2 and rhamnosenegative biotypes are highly pathogenic diarrhea-inducing strains for weaned European rabbits. We describe here the cloning and sequencing of the major subunit gene of a new fimbrial adhesin, adhesive factor/rabbit 2 (AF/R2), which confers on these strains the ability to attach to rabbit enterocytes and to HeLa cells in a diffuse manner and which is associated with in vivo virulence. The chromosomal operon that encodes functional AF/R2 has been cloned from strain B10. The major subunit gene afr2G, as well as an adjacent open reading frame, afr2H, has been sequenced. The Afr2G protein shows homologies with FaeG and ClpG, which are the respective major subunits of fimbrial adhesin K88 (F4) and afimbrial adhesin CS31A. Plasmid carrying the operon transcomplements an AF/R2-negative TnphoA mutant for its ability to express AF/R2. As a whole, AF/R2 is a new member of the *E. coli* K88 adhesin family which is associated with virulence and which may serve in the design of vaccines.

Escherichia coli strains belonging to serovar O103:K-:H2 and to rhamnose-negative biovars are responsible for severe diarrheas in weaned rabbits, with considerable economical involvement in industrial fattening farms from Western Europe (4, 5). Previous data have suggested that these strains are analogous to enteropathogenic E. coli (EPEC) (13): (i) they adhere in vitro to rabbit ileal villi and to HeLa cells in a diffuse pattern by means of a specific adhesin (18), (ii) they induce attachment-effacement lesions in ileal enterocytes of infected rabbits (14, 21), (iii) they possess a gene that is homologous to the *eaeA* gene of EPEC and/or enterohemorrhagic *E. coli* strains (12, 22), and (iv) they do not produce Shiga-like toxins and/or do not have sequences analogous to slt genes (12, 16, 17). The specific adhesin, which is presumably involved in the first step of interaction between bacteria and enterocytes, enables the bacteria to attach to ileal villi of 8-day-old and 6-week-old rabbits, as well as to HeLa cells with a diffuse pattern and in a D-mannose-resistant way (18). A major component of this adhesin may be purified from surface extracts of the strain as a protein with an apparent molecular weight (MW) of 32,000 (32K). This component, as well as antibodies raised against it, inhibits adhesion of bacteria to cells in a competitive way (18). This adhesin is called adhesive factor/ rabbit 2 (AF/R2) to distinguish it from AF/R1 (3), the adhesin expressed by the Rabbit Diarrheal E. coli 1 (RDEC-1) strain (6). Recently, we produced an AF/R2-negative TnphoA mutant derived from the wild-type strain B10 (20). This mutant shows a significantly decreased pathogenicity compared to the wild-type strain when it is administered orally to weaned rabbits, indicating that AF/R2 is an important (although not unique) virulence factor. We also demonstrated that afr2 genetic determinants are carried by the chromosome of O103: K⁻:H2 E. coli strains from rabbits (20).

In this work, we describe the cloning of the whole operon that encodes AF/R2. The 32K major-subunit open reading frame (ORF), *afr2G*, as well as an adjacent ORF, *afr2H*, has been sequenced. Sequence analysis indicates that AF/R2 is a new fimbrial adhesin of *E. coli* belonging to the *fae* (K88 or F4) family.

To clone the afr2 operon, we prepared a genomic library of DNA from the O103:K⁻:H2 and rhamnose-negative E. coli strain B10. Briefly, high-MW DNA was obtained from B10 by lysozyme-proteinase K-Sarkosyl extraction and then by CsCl gradient centrifugation and dialysis (10). Large overlapping fragments were obtained by partial digestion of B10 DNA with Sau3A (Boehringer Mannheim, Meylan, France), size fractioned by a sucrose gradient, and analyzed by pulsed-field gel electrophoresis. Fragments of 35 to 45 kb were ligated to cosmid pHC79 (Boehringer Mannheim) digested by BamHI, packaged in vitro into λ phage capsids (DNA packaging kit; Boehringer Mannheim), and transduced into E. coli HB101. A library comprising 863 ampicillin-resistant and tetracyclinesensitive clones was grown in Penassay broth plus 50 µg of ampicillin per ml and screened for the expression of AF/R2 by dot immunoblotting with anti-32K AF/R2 major subunit antiserum (19). Eight clones were found positive by immunoblotting, and their abilities to produce the 32K AF/R2 subunit were confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting (immunoblotting). Among these, four adhered firmly and in a diffuse manner to the HeLa cell line, as tested by a technique already described elsewhere (18), suggesting that they contained the entire genetic material for active AF/R2 production. The cosmid contained in one clone, named pFMBP5, was purified and further submitted to partial digestion by Sau3A, ligated into BamHI-digested pUC19 (Gibco-BRL, Cergy, France), and transformed into E. coli XL1 Blue. Transformants were selected for their white phenotype on LB agar containing 100 µg of ampicillin and 40 μg of 5-bromo-4-chloro-3-indolyl-β-Dgalactopyranoside (X-Gal) per ml and then screened for expression of AF/R2 as described above. Thirteen independent

^{*} Corresponding author. Mailing address: Unité Associée INRA/ ENV de Microbiologie Moléculaire, Ecole Nationale Vétérinaire, 23 chemin des Capelles, F-31076 Toulouse cedex 3, France. Phone: 33 5 61 19 38 84. Fax: 33 5 61 19 39 75.

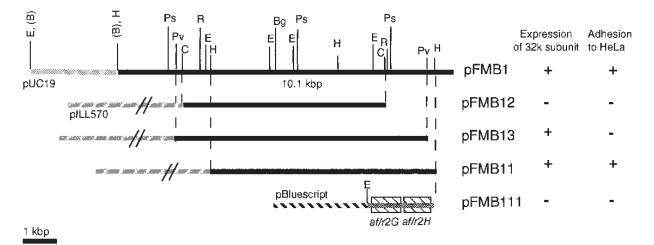


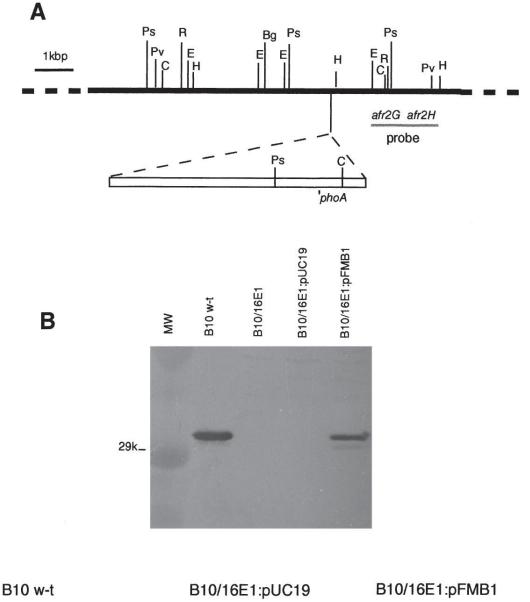
FIG. 1. Map of plasmid pFMB1 and its derivatives. Expression of the 32K AF/R2 subunit was checked in hot urea surface extracts of the strains by SDS-15% PAGE followed by Western blotting with a rabbit anti-32K antiserum (18). Adhesion to HeLa cells was tested as described elsewhere (18). Boxes in pFMB111 show location of ORFs *afr2G* and *afr2H*. The insert of pFMB111 was used as a probe to localize the Tn*phoA* insertion site in the AF/R2-negative B10/16E1 mutant (see Fig. 3). E, *Eco*RI; B, *Bam*HI; Ps, *Pst*I; Pv, *Pvu*II; C, *Cla*I; P, *Eco*RV; H, *Hind*III; Bg, *BgI*II.

subclones of pFMBP5 were found to express the AF/R2 32K subunit, 11 of which adhered to HeLa cells. The sizes of inserts in these plasmids ranged from 10 to ca. 33.6 kb. The smallest plasmid that expressed functional AF/R2, named pFMB1, was selected for further studies. A restriction endonuclease map of pFMB1 is given in Fig. 1. Different fragments of the insert of

pFMB1 were subcloned into the pILL570 (*Not*I) vector (a derivative of pILL570 with an extra *Not*I site and an *Eco*RI site in the polylinker [a kind gift from A. Labigne-Roussel, Institut Pasteur, Paris, France]) (11). None of the *Eco*RI fragments obtained by total or partial digestions or of the individual *Hind*III fragments allowed the expression of AF/R2. However,

ECORI	afr2G	
<u>gaalte</u> ttye gaalageact atgeetgete aggagigatt tigtgalaag teetgaagge tialeaatty taeaaagtet ettiaaatat tgalaaaaag gaaatette		
	Met ly	
AAA CTA ATT GET ATT GET ETA GEA ACA GET TEC OTE ATG TET GGA GTG GEG AAT GET GAG TEA TET TGG GTA GAG GEA TEC AET GGE GGG		
lys leu ile ala ile ala val ala thr ala ser val met ser gly val ala asn ala glu ser ter try val glu ala ser thr gly gly		
ATC GGC GGA ACT ATT GAA GTC GAT AGC CAG TAC GAC GAT CIT TGG ACG TGG AAG TTA GGG GAC GCC ATT ACG GTG GCC AGT AAT GCT GCA		
ile gly gly thr ile glu val asp ser gln tyr asp asp leu trp thr trp lys leu gly asp ala ile thr val ala ser asm ala ala		
GCA GAA AAA ACT TOG CTG ACA ATT ACT ATG GAA CAG CGT AAG CCO CIT CTG GTA GGT AAG TOT GAG CCA TIT AAA GOT CCG TCC ACG GGT		
als glu lys thr ser leu thr ite thr met glu gln arg lys pro leu leu val gly lys ser glu pro phe lys als pro ser thr gly	val gl	y ala
TCG CCG AGT ATT TCT TTC AGT GAC GCT GAC GGG AAT GAA GTT GCT CTT CAA ACC CCT GCC ACC CCA AGC CAG GGC AAG GCA AGT CTG ACG		
ser pro ser ile ser phe ser asp ala asp gly asm glu val ala leu gln thr pro ala thr pro ser gln gly lys ala ser leu thr AAA GAT GAA TCA AAG GCT ACT ATC GGT TCT CTG ACA TTA AAC GTC CAG GCT ATC GGT GTA ATG TAT ACA CAA AGC ACC AAT CCC TCG TAT		
Add GAT GAA TCA AAG GOT ACT ATC GOT TOT OLG ACA TTA AAC GTC CAG GOT ATC GGT GTA ATG TAT ACA CAA AGC ACC AAT CCC TCG TAT lys asp glu ser iys ala thr ile gly ser leu thr leu asn val gln ala ile gly val met tyr thr gln ser thr asn pro ser tyr		
The apply and set the and the the gry bet red the red and we gill and the gry val met for for far har deg for har deg den har for gre and and the gry val met for gre and the gro set vyr		
his ala met thr ala asn asp val gly asn ile phe his gly gly leu pro thr ser ala ala gly leu asn gly ser thr ala val asn		
AGC GAT GGC GGC TGG ACT GGA ATG ANT AA TAA TIG AAT GAA TIG AAT TAA TIG AAT AA TIG ACT GGT CAG AAT AAG TCC TIG ATT CAG AAT CAG ACT GAT CAG AAT AAG TCC TIG ATT CAG AAT AAT TIG ATT AAT AAT TIG AAT		
ser as gly gly trp thr val thr glu met met asn lys trp asn glu phe thr gly gln asn lys ser trp ile gln thr val try ser		
AGC GET AGS ATT GAT GAT GAT THAC ANT TAC AGC GRE TOT ATC GCT CAA GGC CAA AGG GTT GAT GGA GTC GAT TCA CGG GTA AGC ACT		
ser gly chr ile asp ala leu Lyr asn tyr ser leu ser ile ala gln gly gln thr leu glu ala glu phe asp ser pro val thr chr		
TGG AST GCT CCG CTA ACT ATT TCA ATC GCA TAT AAC ta ttigtatget thagecost tectaatgga atggggegtt aacggaatt tatacattga		
trp ser ala pro leu thr ile ser ile ala tvr asn ***	ugenes	
afr2H		
	CAT GI	A AAG
occtgttaaa tgatgtgoot aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG		
	his va	l lye
cootgttaaa tgatgtgoot aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTC AAA ATC TTA ATC CTA AAT TCA GTT TTA TTT CCA GTT TGG Met asn val lew lys ile lew ile ser val lew phe pro val trp	his va CAA AC	l lya C TGG
coctgitaaa tgatgigeet aaaaaaactt tiggggigit aaa ATG AAT GTG TTC AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Met asn val leu lys ile leu ile ser ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA ATA TTC AAT GGT TTC GTT ACC GAT GAG GCA CCA AAA T3G ACA TGG CAA GTT GCT TCA CAT GAT	his va CAA AC gln th	l lys C TGG r trp
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Met am val leu lys ile leu ile aer ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TTC AAT GGT TTC GTT ACC GAT GAG GCA CCA AAA TGG ACA TGG CAA GTT GCT TCA CAT GAT ala glu ile leu amp gly gly glu ile ile phe aen gly phe val thr amp glu ala pro lym trp thr trp gln val ala mer him amp	his va CAA AC gln th TTA TI	l lys C TGG x trp T GAG
cocctgttaaa tgatgtgeet aaaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TG3 Met aan val lew lys ile leu ile eer ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TAT ATC TAT GGT TTC GTT ACC GAT GAA GAA TG3 ACA TGG CAA GTT GCT TCA CAT GAT ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val ala ser his asp AGA GTA GAT ACC GCC AAT GCG CAT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTG 60G CAT	his va CAA AC gln th TTA TT leu ph	l lys C TGG x trp T GAG e glu
coctgttaaa tgatgtgcot aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Met aan val leu lys ile leu ile leu ile ser ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TTC AAT GGT TTC GTT ACC GAT GAG GCA CCA AAA TGG ACA TGG CAA GTT GCT TCA CAT GAT ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aap glu ala pro lys trp thr trp gln val ala ser his asp AGA GTA GAT GCC GCT AAT GCG CGT ATT GAG AAC AAA GAA TTA GTG TTT ATT GG CGC GAT AAA GGT TTT CTG CCT TTT GTG GGC GAT arg val asp thr ala asn ala arg ile glu asn lys glu leu val phe asn leu arg asp lys gly phe leu pro phe leu glu gly his	his va CAA AC gln tř TTA TI leu př AAT TC	l lys C TGG r trp T GAG e glu A CAA
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Met asn val leu lys ile leu ile leu ile ser ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TTC AAT GGT TTC GTT ACC GAT GAG GCA CCA AAA TGG ACA GTT GGT TCA CAT GAT ala glu ile leu asp gly gly glu ile ile phe asn gly phe val thr asp glu ala pro lys trp thr trp gln val ala ser his asp AGA GTA GAT ACC GCC AAT GGG GGT AAT GGT ATT GAG AAA GAA TTA GTG TTT ATT CGA GTT GGT GCT TTT CTT GAG GGG GAT arg val asp thr ala asn ala arg ile glu asn lys glu leu val phe asn leu arg asp lys gly phe leu pro phe leu glu gly his ATA GCC GAA CGT GGC GGG CCT GGT TTT ACT CCG TAC ATT ACG TTC AGC AGT AAT GGA AAG CCT TTT ACG ATA CTC GAA GGG AAG GGT AGC	his va CAA AC gln th TTA TI leu ph AAT TC asn se	l lys C TGG r trp T GAG e glu A CAA r gln
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Met aan val leu lys ile leu ile leu ile ser ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGA ATA ATA TAT TTC AAT GGT TTC GTT ACC GAT GAG GCA CCA AAA TGG ACA TGG CAA GTT GCT TCA CAT GAT ala glu ile leu aap gly gly glu ile ile phe aan gly phe val thr aap glu ala pro lys trp thr trp gln val ala ser his aap AGA GTA GAT ACC GCC AAT GCG CGT AAT GGA ACAA AAA GAA TTA GTG TTT ATG GC GCG GAT AAA GGT TTT CTG CCT TTT CTT GAG GGA CAT arg val aap thr ala aan ala arg ile glu aan lys glu leu val phe aan leu arg aap lys gly phe leu pro phe leu glu gly his ATA GCC GAA CGT GGC GGG CCT GGT TTT ACT CCG TAC CAT ACG TTC AGC AGT AAT GGA AAA GCT TTT ACG ATA CTC GAA GGG AAA GGT AGC ile ala glu arg gly gly pro gly phe thr pro tyr ile thr phe ser ser aan gly lys pro phe thr ile leu glu gly lys gly ser CAT TTT GCG GCA ACA GCT GCG GTA AAA ACT ACT GAC AAT AGT CTA GAA GCT ATT GCT GAT GCT GAA GGT GCT GCT GTT GCT GTT ACG GCT AAT GGA AAA GTT ATT GGC GCA ACA GTT GCT GCA GGA CTT GCT GCT ACC his phe arg ala thr val pro val aan aan pro aap aan aan glu leu ile gly gln leu ala phe aap val aap gln gly leu ala ile	his va CAA AC gln tř TTA TT leu pk AAT TC asn se AGT GT ser va	l lys C TGG r trp T GAG e glu A CAA r gln G GGC 1 gly
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGA Met aan val leu lys ile leu ile aer ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TTT ATA GGT TTT CTG FT ACC GAT GAG GCA CCA AAA TGG ACA TGG CAA GTT GCT TCA CAT GAT ala giu ile leu asp gly gly glu ile ile phe aen gly phe val thr aep glu ala pro lys trp thr trp gln val ala eer his aep AGA GTA GAT ACC GCC AAT GCG CGT AAA ATA ATA TTG GAG AAC AAA GAA TTA GTG TTT AAT CTG GCG GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aer ala arg ile glu aen lye glu leu val phe aen leu arg aep lys gly phe leu pro phe leu glu gly phi ATA GCC GAC GGT GGC GGC CGT GTT TTA CT CCG TAC ATT ACG TTC ACG CAT ATA GGA CTT TTT ACG ATA CTC GAA GGT AGG GAG AGC ile ala glu arg gly gly pro gly phe thr pro tyr ile thr phe ser ser aen gly lys pro phe thr ile leu glu gly lys gly ser CAT TTT GCC GCA ACT CCG GTA AAC AAT CCT GAC AAT AAT GAA CTT ATT GGT CAG CTT GAT GET GAT CAA GGT GCT GAT GAA GGT GTT GAT GGT AGG his phe arg ala thr val pro val aen and pro aep aen aen glu leu ile gly gln leu ala phe aep val aep gln gly leu ala ile AGT CAA GAA GAA FAFT GTC CTC TTT GCC ACA GGT ATT ATT ATT AGG GAA GAA GAT ATT GAT CAC CAA GAT TAT GAT ATC CCA GTT GCT GCT GTT GCT TTT GCC GAC ATT AGT TTA GAA CTT ATT GGT CAG CTT GAT GAT GAT GAA GAT ATT GGT CAC his phe arg ala thr val pro val aen aen pro aep aen aen glu leu ile gly gln leu ala phe aep val aep gln gly leu ala ile AGT CAA GAA GAA FAFT GTT CTT TTT GCC ACA GGT ATTA TTA AGT CTC CCC AAGG	his va CAA AC gln th TTA TT leu ph AAT TC asn se AGT GT ser va GAG GT	l lys C TGG r trp T GAG e glu A CAA r gln G GGC 1 gly G GTT
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGA Met aan val lew lys ile leu ile aer ser val leu phe pro val trp det aan val lew lys ile leu ile aer ser val leu phe pro val trp ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val ala eer his aep AGA GTA GAT ACC GCC AAT GCG CGT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aan ala arg ile glu aen lye glu leu val phe aan leu arg aep lys gly phe leu pro phe leu glu glu glu gly phe ATA GCC GAA CGT GGG CGG CGT GAT TTT ACT CCG TAC ATT ACG TTC ACG TTC AGG AAA GGT TTT AGG ATA CCT GAA GGG AAA GGA AGG TAGG arg val aep thr ala aan ala arg ile glu aen lye glu leu val phe aan leu arg aep lys gly phe leu pro phe leu glu gly phe ATA GCC GAA CGT GGG CGG CGT GGT TTT ACT CCG TAC ATT ACG TTC ACG AGT AAT GGA AAA GGT TTT AGG ATA CTC GAA GGG AAA GGA AGG arg gul aep thr ala asn ala arg ile glu aen lye glu leu val phe aan leu arg aep lys gly phe leu pro phe leu glu gly lys gly ser cAT TTT GGC GCA ACA GCT GGG TTT ACT CCG TAC ATT ACG TTC AGC AGT AAT GGA AAA GCT TTT AGG ATA CTC GAA GGG CTT GGT CTT GAT GGA GGG CTT GGT CTT GCT GAC AAA GGA CTT ATT GGT CAA GAA AAA GGT CCG GTA AAA AAA CAA CCT GAC AAA AAT GAA CTT ATT GGT CAG CTG GCG TTT GAT GGT GAA GGT CTT GAT GTT GAT GCT TAC his phe arg ala thr val pro val asn asn pro aep aan aen glu leu ile gly gln leu ala phe aep val aep gln gly leu ala ile aer gln glu aep ser val val leu pro thr gly met ser phe ile ser gly aep ser ile thr aen val asn thr has er leu pro yas	his va GAA AC gln th TTA TT leu ph AAT TC asn se AGT GT ser va GAG GT glu va	l lys C TGG r trp T GAG e glu A CAA r gln G GGC l gly G GTT l val
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGA Met aan val lew lys ile leu ile leu ile aer ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TAT ATC ATA GGT TTC GTT ACC GAT GAG GAG CCA AAA TGG CAA GTT GCT TCA CAT GAT ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val ase rhis aep AGA GTA GAT ACC GCC AAT GCG CGT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aen ala arg ile glu aen lys glu leu val phe aen leu arg aep lys gly phe leu pro phe leu glu gly his ATA GCC GAA CGT GGC GGG CCT GGT TTT ACT CCG TAC ATT ACG TTC AGC AGT AAT GGA AAA GCA ATA GGA TAT CGG GAA AGT ACC CAT TTT CGC GGC ACG GGG CCT GGT TTT ACT CCG TAC ATT ACG TTC AGC AGT AAT GGA AAA CCT TTT ACG ATA CTC GAA GGG AAA GGT AGC CAT TTT CGC GCA ACA GTT CCG GTA AAC AAT CCT GAC AAT AAT GAA CTT ATT GGT CAG CTG GCG TTT GAT GZT GAT CAA GGT CTT CCT ACC his phe arg ala thr val pro val asn asn pro aep aan aen glu lew ile gly gln lew ala phe asp val aep gln gly lew ala ile AGT CAA GAA GAT AGT GTC GTC TTG CCA ACA GGT ATTG GTA TTA TAT AGC GGA CAAT AAT GAC ATT ATT ATA ACA CAT ACT CAC AAT ACA CAT AGT CTC CCC AAA AGC CGT CAG GAA GAT AGT GTC GTC TTG CCA ACA GGT ATTG GTA TTA TATA ACG CGAG CAT ATC ACC ACA CGT CTC GCC ACT his phe arg ala thr val pro val asn asn pro aep aan aen glu lew ile gly gln lew ala phe asp val aep gln gly lew ala ile AGT CAA GAA GAT AGT GTC GTC TTG CCA ACA GGT ATTG GTA TTA TATA ACG CGAG CAAT ACA CCT AAT ACA CAT AGT CTC CCA AAA AGC CGT CTG TCT GCA CTG GTA ATA ATA AAAT AAA TATA AGA CAAT AGT ACT CAC ACA CGT CTG CTC ACT AGT CAA GAA GAT AGT GTC GTC TTG CCA ACA GGT ATTG GTC ATT TATA AGC GGA GCA AGT ATC ACC ACA CGT CAT ACT CAC ACA GGT ATTG CCA CAC GGT ATTG GCA CCT GTG TCT CCC AAA AGC CGT CTG TCT GCA CTG TTA TTG ATTA AAAT AAAT	his va CAA AC gln ch TTA TT leu ph AAT TC asn se AGT GT ser va GAG GT glu va CTG GC	1 lys C TGG r trp T GAG e glu A CAA r gln G GGC 1 gly G GTT 1 val G AAT
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGA Met asn val leu ly sile leu ile aer ser val leu phe pro val trp det asn val leu ly sile leu ile aer ser val leu phe pro val trp ala glu ile leu asp gly gly glu ile ile phe aen gly phe val thr aep glu ala pro lys trp thr trp gln val ala ser his asp AGA GTA GAT ACG GCC AAT GGG CGA ATA ATA TAT GGA AAC AAA GAA TTA GGG TTT AAT GTG GCA GAT AAA GGT TTTC GT GCT TTT CTT GAG GGG CAT arg val aep thr ala asn ala arg ile glu aen lye glu leu val phe asn leu arg asp lys gly phe leu pro phe leu glu gly phe ATA GCC GAA CGT GGC GGC GCT GGT TTT ACT CCG TAC ATT ACG TTC AGT TTC AGC ATT GGG CAG TTT AAT GGG GGG GAT ATA GCC GAA CGT GGC GGG CGC GGT TTT ACT CCG TAC ATT ACG TTC AGC TTC AGT GAA AGA CTT ATT GGG GGG GGG ile ala glu arg gly gly pro gly phe thr pro tyr ile thr phe ser ser asn gly lys pro phe thr ile leu glu gly lys gly ser CAT TTT CGC GCA ACA GGT CCG GTA AAC AAT CCC GAC AAT AAT GAA CTT ATT GGT CAG CTT GAT GAT GAA GGT TTT GAT GAA GGT CTT GAT GAT GAA GAT AGT GTA GAA GAT AGT GTA GAA GA	his va CAA AC gln th TTA TT leu ph AAT TC asn se AGT GT glu va CTG GC leu al	1 lys C TGG r trp T GAG e glu A CAA r gln G GGC 1 gly G GTT 1 val G AAT a asn
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGA Met aan val lew lys ile leu ile aer ser val lew phe pro val trp det aan val lew lys ile leu ile aer ser val lew phe pro val trp ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val ala eer his aep AGA GTA GAT ACC GCC AAT GCG GOT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aer ala arg ile glu aen lys glu leu val phe aern leu arg aep lys gly phe leu pro phe leu glu gly phe ATA GCT GAC ACC GCC GAT GCG GOT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTT GAG GAG GAG GAT arg val aep thr ala aer ala arg ile glu aen lys glu leu val phe aern leu arg aep lys gly phe leu pro phe leu glu gly yle ser cat TTT CGC GAA CGT GCG GGC GGT ATT GAG AAC AAA GAA TTA GTG TTC AGC AGT AAG GCT TTT ACG ATA CGC GAA GGG AAG GGT ACC ile ala glu arg gly gly pro gly phe thr pro tyr ile thr phe ser ser aern gly lys pro phe thr ile leu glu gly lys gly ser CAT TTT CGC GCA ACA GTT CCG GTA AAC AAT CCT GAC AAT AAT GAA CTT ATT GOT CAG CTG GCG TTT GAT GAT CAA GGT CTT GCT ATC his phe arg ala thr val pro val aen aen pro aep aen aen glu leu ile gly gln leu ala phe aep val aep gln gly leu ala ile ser gln glu aep ser vai val leu pro thr gly met ser phe ile ser gly aep ser ile thr aen val aen thr his ser leu pro ya AGC CTT CTG CT GCA CTG GTTA TTG ATG AAT AAT AAA AAT GGC ATG AGT TA TC CCA AAT ACT GCT CAA CAA GGT GTT ser arg leu ser ala leu leu met aen aen lys phe gly aen gly met ser ser ser ear aen gly his val ile ser gln gly val GGC CAG GTT ACT AAT ATT GCT GCG CAT TAT GCA TTT ACA ATT CAA AAT GGC ATG ACT CAA TAT ACT CCA GCG GAA AAA CAC CCC CCG GCT CAA GGT GTT ser arg leu ser ala leu leu met aen aen lys phe gly aen gly met ser ser ser ear aen gly his val ile ser gln gly val GGC CAG GTT ACT AAT ATT GCT GCG CA TAT GCA TTT TACA AAT GCA TTT ACA ACT CAA GCA TTA CCA GCG GAA AAC ACC CCC GCG CCA TAG	his va CAA AC gln th TTA TI leu ph AAT TC asn se AGT GI ser va GAG GI glu va CTG GC Leu al GAA GC	1 lys C TGG r trp T GAG e glu A CAA r gln G GAA f GGL G GL 1 glL G GTT 1 val G AAT a asn T AGT
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Mat aan val lew lys ile leu ile aer ser val leu phe pro val trp Mat aan val lew lys ile leu ile aer ser val leu phe pro val trp ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val ala eer his asp AGA GTA GAT ACC GCC AAT GCG COT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aan ala arg ile glu aen lye glu leu val phe aan leu arg aep lys gly phe leu pro phe leu glu gly phe ATA GCC GAA CGT GGG CGG GGT GTA TAC GGT TTA CCC GAC AAT AGG ATT AGG TAT AGG ATA GGA AAG GAT TTT GGG CGC TTT ACG ATA GGA GAA GAA GAA arg val aep thr ala aen ala arg ile glu aen lye glu leu val phe aen leu arg aep lys gly phe leu pro phe leu glu gly jks ATA GCC GAA CGT GGG CGG GGT GTT TACT CCG TAC ATT AGG TTC AGC AGT AAT GGA AAA GAA AGGT TTT AGG ATA GGA GAA GGT GGC GGG CTT GAT GAA GGA AAG GAT TTT AGG TAC GAT AAG GAT AAT GAA CTT ATT GGG CAA AAA GGT CTG GAA GGT CTT GCT AGC ATA AGT CAA GAA GAT AGT GCC GGG CTG GTA AAC AAT CCT GAC AAT AAT GAA CTT ATT GGT CAG CTG GCG TTT GAT GAT GAA GGT CTT GCC AAG AGT CAA GAA GAT AGT GCC GTC TTG CCA ACA GGT ATG TCA TTT ATA AGC GGA GAC AGT ATC AGC GAA GGT CAT AGT CCC CAA AGG AGC CGT CGG TCT GCC ATG CTT TTG CAA AGA GTA TTT ATA AGA CTT ATT GGA AAC GCT AAT GGC CAC GTA ATC AGT CCC AAG AGC CGT CGG TCG GCG CTG GTT ATTG ATG AAT AAA TTT GGA AAT GGC AGT AGT ACC ACG GTC AAT ACA CAT AGT CCC CAA GGG AGC CGG CGG CCG GTC GTG TTA TTG ATG AAT AAA TTT GGA AAT GGC ATG AGT TCA TCC AGT AAT GGC CAC GTA ATC AGT CAG GGT GTT ser arg leu ser ala leu leu met aan aan lys phe gly aen gly met ser ser ser ser ser ser aan gly his val ile ser gln gly val GGC CGA GTT CGG GTA AAT AAT GGG GCA TAT GCA CTT AGT TTA AGC GGA GTT CAA TTA CAG GGG AAA CAC CCC GGG CG CG CAA TAG giy gln val thr asn ile ala ala ala tyr ala ser ser leu ser aen phe glu aen thr pro ala arg trp	his va CAA AC gln th TTA TI leu ph AAT TC asn se AGT GI ser va GAG GI glu va CTG GC Leu al GAA GC	1 lys C TGG r trp T GAG e glu A CAA r gln G GAA f GGL G GL 1 glL G GTT 1 val G AAT a asn T AGT
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGA Met aan val lew lys ile leu ile leu ile aer ser val leu phe pro val trp GCT GAA ATA CTT GAT GGT GGT GAA ATA ATA TA TTC ATA GGT TTC GTT ACC GAT GAG GAG CCA AAA TGG CAA GTT GCT TCA CAT GAT ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val aer his aep AGA GTA GAT ACC GCC AAT GCG CGT ATT GAG AAC AAA GAA TTA GTG TTT ATG CTG GCG GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aen ala arg ile glu aen lye glu leu val phe aen leu arg aep lye gly phe leu pro phe leu glu gly his ATA GCC GAA CGT GGG GGG CT GGT TTT ACT CCT GAC ATT ACG TTC AGC AGT AAT GGA AAA GCT TTT ACG ATT CTG GAA GGG AAA GGT AGC CAT TTT CGC GGC ACG GGG CT GGT TTT ACT CCT GAC AAT AAT GAA CTT ATT GGT CAG CTG GCG TTT GAT GTT GAT GGA GGT CTT GCT ATT his phe arg ala thr val pro val asn asn pro aep aan aen glu lew ile gly gln lew ala phe aep val aep gln gly lew ala ile AGC CGT CTG GCT GGC CTG GTC TTG CCA ACA GGT ATT GGC ATT ATA GGC AGT ACC ACA CGT CAC ACT ACT CCC CCA AGA AGC GGT CTG GCT GGC CTG GTC TTG CCA ACA GGT ATT GTG TA TTA TA ACC GGA GAC AGT ACC ACC GCT CAT CAA GGT CTT CCC CCA AGA AGC CGT CAG GAA GAT AGT GTC CTT GCCA ACA GGT ATG GCA TTA TAT AGC CAG AGT ACC ACC GTC ACT AAT GGC CCA CTG ATT ACG TTG CCC CCA AGA AGC CGT CTG TCT GCA CTG TTA CTG AAA AAT AAT GTA GCC AGT AGT ACC ACC GCC GTA ATC AGT CAC CCC GCG GTT ser arg lew ser ala lew lew met aan ann lys phe gly aen gly met ser ser ser ser ser aen gly his val ile ser gln gly val GGC CAG GTT ACT AAT AAT ATT GCT GCA TTA TAT ACCA AGT TTT GAG GTT CGA CTG GGA AAA ACA CCC CCG GCT CAT TGG GGT CAG GTT ACT AAT ATT GCT GCT ACT AFT ATCA AAT TTT GAG CTT CGA TTA CCA GGG GAA AAC ACC CCG GCT CTA GGT CAG GTT ACT AAT ATT GCT GCA TTA TTT GGA AAT GGC ATG AGT ACT CCA GGG GAA AAC ACC CCG GCT CAT TGG GC CAG GTT ACT AAT ATT GAT GAT AAT AAT TTT GGA AAT GGC ATG GAT TCA ACT CCA GGG GAA AAC ACC CCG GCT CA TGG GJy gln val thr aen ile ala ala ala tyr al	his va CAA AC gln th TTA TI leu ph AAT TC asn se AGT GI ser va GAG GI glu va CTG GC Leu al GAA GC	1 lys C TGG r trp T GAG e glu A CAA r gln G GAA f GGL G GL 1 glL G GTT 1 val G AAT a asn T AGT
coctgttaaa tgatgtgeet aaaaaaactt ttggggtgtt aaa ATG AAT GTG TTG AAA ATC TTA ATC CTA ATT TCA TCA GTT TTA TTT CCA GTT TGG Mat aan val lew lys ile leu ile aer ser val leu phe pro val trp Mat aan val lew lys ile leu ile aer ser val leu phe pro val trp ala glu ile leu asp gly gly glu ile ile phe aan gly phe val thr aep glu ala pro lys trp thr trp gln val ala eer his asp AGA GTA GAT ACC GCC AAT GCG COT ATT GAG AAC AAA GAA TTA GTG TTT AAT CTG CGC GAT AAA GGT TTT CTG CCT TTT CTT GAG GGG CAT arg val aep thr ala aan ala arg ile glu aen lye glu leu val phe aan leu arg aep lys gly phe leu pro phe leu glu gly phe ATA GCC GAA CGT GGG CGG GGT GTA TAC GGT TTA CCC GAC AAT AGG ATT AGG TAT AGG ATA GGA AAG GAT TTT GGG CGC TTT ACG ATA GGA GAA GAA GAA arg val aep thr ala aen ala arg ile glu aen lye glu leu val phe aen leu arg aep lys gly phe leu pro phe leu glu gly jks ATA GCC GAA CGT GGG CGG GGT GTT TACT CCG TAC ATT AGG TTC AGC AGT AAT GGA AAA GAA AGGT TTT AGG ATA GGA GAA GGT GGC GGG CTT GAT GAA GGA AAG GAT TTT AGG TAC GAT AAG GAT AAT GAA CTT ATT GGG CAA AAA GGT CTG GAA GGT CTT GCT AGC ATA AGT CAA GAA GAT AGT GCC GGG CTG GTA AAC AAT CCT GAC AAT AAT GAA CTT ATT GGT CAG CTG GCG TTT GAT GAT GAA GGT CTT GCC AAG AGT CAA GAA GAT AGT GCC GTC TTG CCA ACA GGT ATG TCA TTT ATA AGC GGA GAC AGT ATC AGC GAA GGT CAT AGT CCC CAA AGG AGC CGT CGG TCT GCC ATG CTT TTG CAA AGA GTA TTT ATA AGA CTT ATT GGA AAC GCT AAT GGC CAC GTA ATC AGT CCC AAG AGC CGT CGG TCG GCG CTG GTT ATTG ATG AAT AAA TTT GGA AAT GGC AGT AGT ACC ACG GTC AAT ACA CAT AGT CCC CAA GGG AGC CGG CGG CCG GTC GTG TTA TTG ATG AAT AAA TTT GGA AAT GGC ATG AGT TCA TCC AGT AAT GGC CAC GTA ATC AGT CAG GGT GTT ser arg leu ser ala leu leu met aan aan lys phe gly aen gly met ser ser ser ser ser ser aan gly his val ile ser gln gly val GGC CGA GTT CGG GTA AAT AAT GGG GCA TAT GCA CTT AGT TTA AGC GGA GTT CAA TTA CAG GGG AAA CAC CCC GGG CG CG CAA TAG giy gln val thr asn ile ala ala ala tyr ala ser ser leu ser aen phe glu aen thr pro ala arg trp	his va CAA AC gln th TTA TI leu ph AAT TC asn se AGT GI ser va GAG GI glu va CTG GC Leu al GAA GC	1 lys C TGG r trp T GAG e glu A CAA r gln G GAA f GGL G GL 1 glL G GTT 1 val G AAT a asn T AGT

FIG. 2. DNA sequence of pFMB111. The deduced amino acid sequences of Afr2G, the major subunit of AF/R2, and of Afr2H are given. The three asterisks indicate stop codons for translation into amino acids. DNA was sequenced by Genome Express (Grenoble, France) by the dideoxy method.



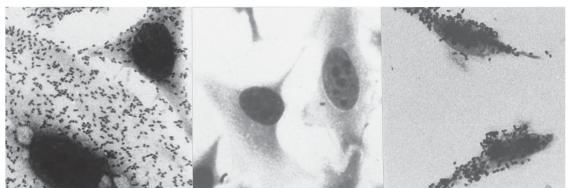


FIG. 3. (A) Insertion site of the transposon TnphoA in the genome of the AF/R2-negative mutant B10/16E1 (20). To localize insertion, the *afr2GH* digoxigeninlabelled probe was used in Southern blots of digestions of B10 and B10/16E1 genomic DNA with *Eco*RI, *Hin*dIII, *Cla*I, *Eco*RV, or *PstI* restriction endonuclease. (B) Transcomplementation of B10/16E1 with pFMB1. The plasmid pUC19 or pFMB1 was transferred into B10/16E1. Transformants were selected by resistance to ampicillin and were then tested for expression of the AF/R2 32K subunit (Afr2G) by SDS-PAGE (not shown) and immunoblotting (upper panel) and for adhesion to HeLa cells (lower panels). MW, prestained MW markers (Gibco-BRL 26041-020); w-t, wild type.

the 6.9-kb HindIII fragment obtained by partial digestion in pFMB11 permitted expression of the 32-kDa subunit as well as adhesion to HeLa cells. Interestingly, the 6.4-kbp ClaI fragment (pFMB12) did not allow expression of the 32K subunit or adhesion of XL1 Blue to HeLa cells, while the 8-kbp PvuII fragment in pFMB13 allowed expression of the 32K subunit but not adhesion to HeLa cells (Fig. 1). This suggested that the gene encoding the major subunit was located near the right end of pFMB11. Therefore, we subcloned the 1.8-kbp EcoRI-HindIII fragment into pBluescript (pFMB111 [Fig. 1]), and both DNA strands were sequenced by the dideoxynucleotide technique. It reveals ORFs, the sequences of which are shown in Fig. 2. Sequence analyses and comparisons were performed on line by use of software developed by the University of Wisconsin Genetics Computer Group (7). The first ORF is 840 bp long (Fig. 2) and encodes a precursor protein of 279 amino acids, with a predicted MW of 28,984 and with a hydrophobic signal sequence of 19 amino acids, giving a mature protein of 260 amino acids with a predicted MW of 27,014. This protein shows an overall identity of 34.3% with the protein FaeG and of 35% with ClpG, which are the respective major subunits of the fimbria K88 (F4) (1, 8) and of the adhesin CS31A (9). It shares with FaeG and ClpG several features that assign them to class 3 fimbria major subunits, as proposed by Low et al.: lack of cysteine residues, penultimate tyrosine at the carboxy terminus, and four conserved prolines in aligned sequences (15). The second 780-bp ORF starts 114 bp downstream of the stop codon of afr2G (Fig. 2). It encodes a precursor protein of 259 amino acids, with a predicted MW of 28,232 and a signal sequence. This protein shows 63.9% identity with the proteins FaeH and ClpH, which are minor components of the adhesins cited above (2, 6a [accession no. M96152]). On the basis of these structural homologies, we called these proteins, respectively, Afr2G and Afr2H.

Recently, an AF/R2-negative mutant called B10/16E1 was produced by single insertion of TnphoA into the wild-type strain B10 (20). This mutant has lost its ability to produce the 32K AF/R2 subunit and to adhere to HeLa cells. It colonizes the digestive tracts of rabbits poorly and is significantly less virulent than the B10 wild-type strain (20). The 1.8-kbp EcoRI-HindIII fragment that contains afr2G and afr2H ORFs was used as a probe, after labelling with digoxigenin (DIG UTP labelling kit; Boehringer Mannheim) as described by the manufacturer, to localize the TnphoA insertion in the mutant B10/ 16E1. In B10/16E1, the transposon is inserted ca. 1.4 kb upstream from the ClaI-EcoRV-PstI sequence inside afr2G (Fig. 3). When it was transformed by plasmid pFMB1, the ability of B10/16E1 to produce AF/R2 and to adhere to HeLa cells was restored (Fig. 3).

Based on the homologies to FaeG and ClpG, it seems very likely that Afr2G is the major component of the AF/R2 adhesin, which, once isolated from surface extract of wild-type O103 strains, migrates in SDS-15% PAGE gels with an apparent molecular mass of 32 kDa (18). The discrepancy between the apparent molecular mass of this kind of protein isolated from wild-type strains and the molecular mass predicted from the sequence is well documented. For instance, the major subunit of AF/R1 was first reported to be 19 kDa (3) but ran to 17 kDa when the operon was expressed in minicells, while the molecular mass deduced from the sequence was calculated to be 14,401 Da (23). The role of Afr2H remains putative, but it may be involved in the adhesive activity of the fimbrial structure, since its absence of production in XL1 Blue:pFMB13 (in which its ORF is truncated) correlates with an absence of adhesion to HeLa cells. The corresponding FaeH protein has been shown to be important in the biogenesis but not in the adhesive

properties of the K88 fimbriae (2). As a whole, our results indicate that AF/R2 is a new member of the K88 family of E. coli adhesins and give new tools to study its involvement in the pathogenesis of EPEC-like strains from rabbits as well as to analyze the regulation of its synthesis and to rationally design vaccines.

Nucleotide sequence accession number. The complete sequence reported in this paper has been assigned GenBank accession no. U77302.

This project was supported by the Conseil Régional Midi-Pyrénées (Toulouse, France).

We thank E. Oswald and J. De Rycke for helpful discussions and critical reading of the manuscript.

REFERENCES

- 1. Bakker, D., P. T. Willemsen, L. H. Simons, F. G. van Zijderveld, and F. K. de Graaf. 1992. Characterization of the antigenic and adhesive properties of FaeG, the major subunit of K88 fimbriae. Mol. Microbiol. 6:247-255.
- 2. Bakker, D., P. T. Willemsen, R. H. Willems, T. T. Huisman, F. R. Mooi, B. Oudega, F. Stegehuis, and F. K. de Graaf. 1992. Identification of minor fimbrial subunits involved in biosynthesis of K88 fimbriae. J. Bacteriol. 174: 6350-6358.
- 3. Berendson, R., C. P. Cheney, P. A. Schad, and E. C. Boedecker. 1983. Species-specific binding of purified pili (AF/R1) from the Escherichia coli RDEC-1 to rabbit intestinal mucosa. Gastroenterology 85:837-845.
- 4. Blanco, J. E., M. Blanco, J. Blanco, L. Rioja, and J. Ducha, 1994. Serotypes. toxins and antibiotic resistance of Escherichia coli strains isolated from diarrhoeic and healthy rabbits in Spain. Vet. Microbiol. 38:193-201.
- 5. Camguilhem, R., and A. Milon. 1989. Biotypes and O serogroups of Escherichia coli involved in intestinal infections of weaned rabbits: clues to diagnosis of pathogenic strains, J. Clin, Microbiol. 27:743-747
- 6. Cantey, J. R., and R. K. Blake. 1977. Diarrhea due to Escherichia coli in the rabbit: a novel mechanism. J. Infect. Dis. 135:454-462.
- 6a.Der Vartanian, M. Unpublished data.7. Devereux, J. P., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387-395.
- 8. 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.
- 9. Girardeau, J. P., Y. Bertin, C. Martin, M. Der Vartanian, and C. Boeuf. 1991. Sequence analysis of the clpG gene, which codes for surface antigen CS31A subunit: evidence of an evolutionary relationship between CS31A, K88, and F41 subunit genes. J. Bacteriol. 173:7673-7683.
- 10. Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow, 1981, Construction and expression of recombinant plasmids encoding type I or Dmannose-resistant pili from a urinary tract infection Escherichia coli isolate. Infect. Immun. 33:933-938.
- 11. Labigne, A., V. Cussac, and P. Courcoux. 1991. Shuttle cloning and nucleotide sequences of Helicobacter pylori genes responsible for urease activity. J. Bacteriol. 173:1921-1931.
- 12. Leroy, S. M., M. C. Lesage, E. Chaslus-Dancla, and J. P. Lafont. 1994. Presence of *eaeA* sequences in pathogenic and non-pathogenic *Escherichia coli* strains isolated from weaned rabbits. J. Med. Microbiol. **40**:90–94.
- 13. Levine, M. M. 1987. Escherichia coli that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. J. Infect. Dis. 155:377–389.
- 14. Licois, D., A. Reynaud, M. Fédérighi, B. Gaillard-Martinie, J. F. Guillot, and B. Joly. 1991. Scanning and transmission electron microscopic study of adherence of Escherichia coli O103 enteropathogenic and/or enterohemor-rhagic strain GV in enteric infection in rabbits. Infect. Immun. 59:3796–3800.
 Low, D., B. Braaten, and M. Van Der Woude. 1996. Fimbriae, p. 146–157. In
- F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), Escherichia coli and Salmonella: cellular and American Society for Microbiology, Washington, D.C.
- molecular biology. American Society for Microbiology, Washington, D.C. 16. Marenda, M., A. Milon, R. Bauerfeind, and M. Boury. 1992. Toxines Shigalike et Escherichia coli entéropathogènes (EPEC-like) du lapin sevré. Rev. Méd. Vét. 143:333-340.
- 17. Mariani-Kurkdjian, P., E. Denamur, A. Milon, B. Picard, H. Cave, N. Lam bert-Zechovsky, C. Loirat, P. Goullet, P. Sansonetti, and J. Elion. 1993. Identification of a clone of *E. coli* O103:H2 as a potential agent of hemolytic and uremic syndrome in France. J. Clin. Microbiol. 31:296-301.
- 18. Milon, A., J. Esslinger, and R. Camguilhem. 1990. Adhesion of Escherichia *coli* strains isolated from diarrheic weaned rabbits to intestinal villi and to HeLa cells. Infect. Immun. **58**:2690–2695.
- 19. Milon, A., J. Esslinger, and R. Camguilhem. 1992. Oral vaccination of weaned rabbits against enteropathogenic Escherichia coli-like E. coli O103 infection: use of heterologous strains harboring lipopolysaccharide or adhe-

sin of pathogenic strains. Infect. Immun. 60:2702-2709.

- vin of pathogenic strains. Infect. Immun. 60:2702–2709.
 Pillien, F., C. Chalareng, M. Boury, C. Tasca, J. De Rycke, and A. Milon. 1996. Role of adhesive factor/rabbit 2 in experimental enteropathogenic *Escherichia coli* O103 diarrhea of weaned rabbit. Vet. Microbiol. 50:105–115.
 Pohl, P., J. E. Peeters, E. R. Jacquemin, P. F. Lintermans, J. G. and Mainil. 1993. Identification of *eae* sequences in enteropathogenic *Escherichia coli* strains from rabbits. Infect. Immun. 61:2203–2206.
- Robins-Browne R. M., A. M. Tokhi, L. M. Adams, V. Bennett-Wood, A. V. Moisidis, E. O. Krejany, and L. E. O'Gorman. 1994. Adherence character-istics of attaching-effacing strains of *Escherichia coli* from rabbits. Infect. Immun. 62:1584–1592.
- Wolf, M. K., and E. C. Boedecker. 1990. Cloning of the genes for AF/R1 pili from rabbit enteroadherent *Escherichia coli* RDEC-1 and DNA sequence of the major structural subunit. Infect. Immun. 58:1124–1128.