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Distribution and Degree of Heterogeneity of the Afimbrial-Adhesin-Encoding Operon (*afa*) among Uropathogenic *Escherichia coli* Isolates

AGNÈS LABIGNE-ROUSSEL[1]* AND STANLEY FALKOW[2]

Unité des Enterobactèries, Institut National de la Santé et de la Recherche Médicale U199, Institut Pasteur, 28 Rue du Docteur Roux, 75015 Paris, France,[1] and Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305[2]

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The afimbrial adhesin (AFA-I) from a pyelonephritic *Escherichia coli* isolate (KS52) is a mannose-resistant, P-independent, X-binding adhesin, expressed by the *afa-1* operon. It is distinct from the *E. coli* X-binding adhesins with M and S specificity. A total of 138 *E. coli* isolates belonging to various serotypes, mostly from urinary tract infections, were screened for the presence of DNA sequences related to the *afa* operon and for the expression of an X-adhesin able to mediate mannose-resistant hemagglutination (MRHA) and adhesion to uroepithelial cells. Fifteen strains were shown to harbor DNA sequences related to the AFA-I-encoding operon, and 13 of them expressed an X-adhesin. Using as probes different DNA segments of the AFA-I-encoding operon in Southern experiments, we demonstrated that only three of these clinical isolates contained genetic determinants closely related to those identified in the original *afa* prototype strain (KS52): presence of the *afaA*, *afaB*, *afaC*, *afaD*, and *afaE* genes associated with the expression of a 16,000-dalton hemagglutinin-adhesin which strongly cross-reacted with AFA-I-specific antibodies. The other *E. coli* isolates harbored DNA sequences homologous to the *afaA*, *afaB*, *afaC*, and *afaD* genes, but lacked the sequence corresponding to the adhesin-producing gene *afaE*; Western blots allowed the detection of polypeptides (15,000, 15,500, or 16,000 daltons) in these strains which cross-reacted with variable intensity with antibodies raised against the denatured AFA-I protein, but did not cross-react with native AFA-I-specific antibodies. Following DNA cloning experiments from chromosomal DNA of two of those strains (A22 and A30), we demonstrated that although the AFA-related operon in A22 and A30 strains lacked the AFA-I adhesin-encoding gene, they synthesized a functional X-adhesin. Thus, strains A22 and A30 encode adhesins designated AFA-II and AFA-III, which were cloned on recombinant plasmids pILL72 and pILL61, respectively. Southern hybridization experiments and Western blot analyses of the 15 AFA-related strains demonstrate the heterogeneity of the genetic sequences encoding the structural adhesin and suggest the bases for the serological diversity of the AFA adhesins.

Escherichia coli strains causing urinary tract infections (UTIs) commonly adhere to uroepithelial cells through bacterial adhesins. Several bacterial adhesins appear to be essential for the pathogenesis of UTIs (33) and to recognize specific cell surface receptors distinct from the α-D-mannose recognized by the type 1 pili (6). These receptors are present on both the uroepithelial and erythrocyte cell surfaces, so that uropathogenic strains often have the ability to clump human erythrocytes in the presence of D-mannose, a property referred to as mannose-resistant hemagglutination (MRHA) (8).

A high percentage of MRHA *E. coli* strains isolated from cases of pyelonephritis (12, 13, 35) encode an operon which expresses both the P pili (10, 19, 24, 25) and an adhesin that specifically recognizes on the host cell surface-globoseries glycolipids which contain the disaccharide α-D-galactosyl-(1,4)-β-D-galactose (14). However, 10 to 15% of the MRHA uropathogenic *E. coli* strains recognize receptors other than the digalactoside (35) and encode mannose-resistant binding specificities which have been referred to as X adhesins (34, 30).

We recently characterized one of these at the molecular level by cloning from the nonfimbriated uropathogenic *E. coli* strain KS52 a 6.7-kilobase (kb) chromosomal DNA fragment (pILL14) (Fig. 1), which was shown to be necessary for host cell MRHA expression and adherence to human transitional and squamous uroepithelial cells (17). The afimbrial adhesin (AFA) does not bind glycophorin A or sialyl glycosides (37) and is therefore distinct from the *E. coli* X-binding adhesins with M (31, 34) and S (16) specificity. This 6.7-kb fragment harboring the *afa* operon expresses five polypeptides with molecular masses of 13,000, 16,000, 18,500, 30,000, and 100,000 daltons encoded by the *afaA*, *afaE*, *afaD*, *afaB*, and *afaC* genes, respectively. Among these, only *afaB*, *afaC*, and *afaE* appeared to be required for the MRHA and adherence expression (18). The *afaE* gene has been identified as the structural gene encoding the hemagglutinin and sequenced (18). It encodes a 131-residue mature polypeptide, the AFA-I adhesin (18, 37), which mediates specific binding to uroepithelial cell and human erythrocyte receptors. The nature of the receptor on the eucaryotic cell surface is not yet known.

In this study we set out to determine whether the genetic information encoded by the *afa* operon identified in a single *E. coli* clinical isolate was commonly found and expressed among other *E. coli* isolates originating from either uropathogenic or fecal specimens. Consequently, we screened 138 strains by colony hybridization for the presence of AFA-I-related DNA sequences and examined the hemagglutinin and adhesive properties expressed by strains having DNA homology with the *afa* operon. The degree of hetero-

* Corresponding author.

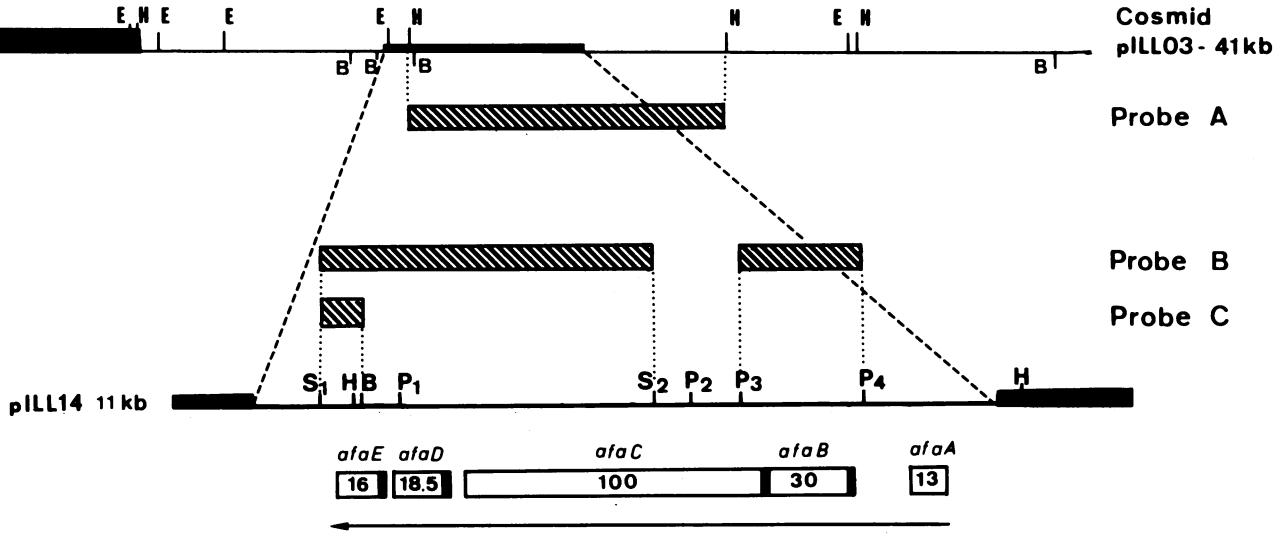

FIG. 1. Linear restriction map of pILL03 and pILL14 hybrid plasmids. pILL14 hybrid plasmid was subcloned from the pILL03 plasmid as previously described (17). Solid bars, pHC79 and pBR322 for the pILL03 and pILL14 maps, respectively; solid lines, DNA inserted in vitro by ligation. Restrictions sites for *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), *Sma*I (Sm), and *Sal*I (S) are shown. The length of the bars corresponds to the length of DNA required to code for the indicated polypeptides. The numbers in the bars and the solid part of the bars indicate the apparent molecular weights of the mature polypeptides (in thousands) and the part of the gene encoding the signal peptide, respectively. This figure summarizes the results previously described (17, 18).

geneity of the related operons expressing X adhesin were investigated.

MATERIALS AND METHODS

Bacterial strains and plasmids. The 138 *E. coli* strains used in this study belonged to sets of clinical isolates provided by R. Welch, G. Kallenius, K. Vosti, and T. A. Stamey and obtained from urine specimens of patients with clinically documented pyelonephritis (15 cases), cystitis (79 cases), or asymptomatic bacteriuria (6 cases) or from fecal specimens isolated from healthy individuals (38 cases). The properties and origins of the *E. coli* strains sharing DNA homology with the *afa* operon and discussed in this study are listed in Table 1. *E. coli* HB101 (1) (*hsdR hsdM recA13 supE44 lacZ4 leuB6 proA2 thi-1* Sm^r) was used as host for all recombinant plasmids involved in this study. The properties of plasmids pILL03 and pILL14 (17, 18) are summarized in Fig. 1.

Bacteria were grown in L-broth without glucose (per liter: 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, pH 7) or on L-agar plates solidified with 1.5% agar (Difco Laboratories).

Receptor binding assays. Hemagglutination, hapten inhibition of hemagglutination by α-methylmannoside (Sigma Chemical Co., St. Louis, Mo.) or α-D-galactosyl-(1,4)-β-D-galactose and latex agglutination assays were performed as described previously (17). These assays allowed the determination of P-receptor or X-receptor-binding specificities and the presence of type 1 pili.

Detection of hemolysin. Hemolysin production was assayed by seeding bacterial cells onto Oxoid CM-55 agar containing 7% defibrinated sheep blood and observing the lysis of erythrocytes after overnight incubation.

Adherence assay. Adherence to uroepithelial cells was tested as described previously (17).

Electron microscopy. Unfixed bacterial suspensions were examined for fimbrialike structures as described previously (17).

Colony hybridization. Bacterial cells grown for 3 h on nitrocellulose filters were used for colony hybridization as described by Grunstein and Hogness (7) with the B probe shown in Fig. 1.

Preparation of DNA. Whole-cell DNA was prepared from a 5-ml L-broth overnight culture. Cells were washed in saline-EDTA (0.5 M NaCl, 0.01 M EDTA, pH 8.0) containing lysozyme (10 mg/ml). After a 30-min incubation at room temperature, cells were allowed to lyse by adding sodium dodecyl sulfate (SDS) (0.8 ml of 10% SDS) and incubated with 100 μl of proteinase K (Merck; 20 mg/ml) for 2 h at 37°C

TABLE 1. Properties expressed by *E. coli* strains harboring DNA sequences related to the *afa* operon

Strain	Sero-type	Source[a]	Type 1 pili[b]	X^{+c}	P^{+d}	Hemolysin	Adhesion[e]
KS52	O2	Pyel	+	+	−	−	+
3669	O2	Pyel	+	+	+	−	+
A11	O6	Pyel	+	+	−	−	+
A43	O1	Cyst	+	+	−	−	+
A2	O15	Cyst	+	+	−	−	+
A22	O15	Cyst	+	+	−	−	+
6584	O4	Cyst	+	+	+	−	+
4006a	O19	Cyst	+	+	−	−	+
A30	O75	Cyst	+	+	−	−	+
A57	O75	Cyst	+	−	−	−	−
3214	O75	Cyst	+	+	−	−	+
A13	O75	Cyst	+	+	−	−	+
7292	O11	Cyst	+	+	−	−	+
6290	O11	Cyst	+	+	−	−	+
7293	O11	Fec	+	+	−	−	+
A77	O133	Fec	+	−	−	−	−

[a] Pyel, Pyelonephritis; Cyst, cystitis; Fec, fecal isolates.
[b] Presence of type 1 pili was demonstrated by mannose-sensitive hemagglutination of guinea pig erythrocytes and electron microscopic analysis after growth in liquid culture.
[c] Strains were determined to encode an X-adhesin when they were capable of agglutining both P1 and p^- human erythrocytes in the presence of D-mannose (6).
[d] Presence of P-specific adhesin was demonstrated by agglutination of synthetic digalactoside-absorbed latex beads (17).
[e] Adhesion to uroepithelial cells was performed as previously described (17).

or overnight at room temperature. Proteins were removed by two successive phenol-chloroform extractions; the viscous DNA was then extensively dialyzed against TE buffer (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA) at room temperature for 12 h, followed by dialysis at 4°C for 36 h.

Plasmid DNA was isolated by an alkaline lysis procedure (21).

Cosmid cloning. Chromosomal DNA from *E. coli* A22 was partially cleaved with the restriction endonuclease *Sau*3A, sized on sucrose gradients (10 to 40%), and ligated to the *Bam*HI-digested and alkaline phosphatase-treated cosmid vector pHC79 (4) as described previously (21). Cosmids were packaged into phage lambda particles (10) and used to infect *E. coli* HB101.

Hybridization. Whole-cell DNA was restricted by various restriction endonucleases, fractionated by agarose gel electrophoresis (0.7%), transferred to nitrocellulose sheets (0.45 μm; Scheicher & Schuell, Inc., Keene, N.H.) by the Southern technique (32), and hybridized (5) with probes labeled with ^{32}P-labeled deoxyribonucleotides (New England Nuclear Corp., Boston, Mass.) by nick translation (22). The specific restriction endonuclease DNA fragments used as probes (A, B, and C, Fig. 1) were isolated and purified as described previously (17). Hybridization was revealed by autoradiography with XAR-Omat (Kodak) films in the presence of an intensifying screen for various lengths of time at −70°C.

Antibody production. Antiserum against the denatured hemagglutinin AFA-I protein was raised as described previously (17), and antiserum against the native AFA-I protein was kindly provided by A. Schmidt (37).

Western blotting. Western blotting (immunoblotting) after SDS-polyacrylamide gel electrophoresis (PAGE) of whole-cell extracts was performed as described (3). Diluted (1:1,000) antisera raised against the denatured AFA-I protein or the native protein purified from HB101 harboring the pILL14 plasmid was used.

RESULTS

Screening for the presence of *afa*-related sequences among *E. coli* isolates. A total of 138 *E. coli* strains originating from patients with pyelonephritis, cystitis, or asymptomatic bacteriuria or from fecal specimens of healthy individuals were grown on nitrocellulose filters and screened by colony hybridization for the presence of sequences homologous to that of the *afa* operon with a probe (probe B, Fig. 1) overlapping the *afaB*, *afaC*, *afaD*, and *afaE* genes. A positive signal was obtained with 2 of the 15 pyelonephritic strains (13%), 11 of the 79 cystitis strains (14%), none of the 6 asymptomatic bacteriuria strains, and 2 of the 38 fecal strains (5%) (Table 1).

Characteristics and properties expressed by the strains harboring *afa*-related sequences. The 15 strains having DNA with fragments internal to the 6.7-kb pILL14 insert encode lipopolysaccharide antigens characteristic of various serotype groups (Table 1). None of them produced a hemolysin, but all of the strains expressed mannose-sensitive hemagglutination of guinea pig erythrocytes consistent with the presence of type 1 pili on their cell surface. In addition, 13 of the 15 isolates exhibited D-mannose-resistant hemagglutination for fresh human blood cells, including erythrocytes belonging to the p$^-$ blood group [i.e., lacking the α-(1,4)-digalactoside]; this result indicated that, except for strains A77 and A57, all the isolates sharing DNA sequences related to the *afa* operon expressed an MRHA adhesin with an independent α-(1,4)-digalactoside-binding specificity, i.e., an X adhesin.

This phenotype correlates with the ability of these strains to adhere to uroepithelial cells (Table 1). The *E. coli* strains 6584 and 3669 were the only ones capable of mediating agglutination of the synthetic digalactoside-adsorbed latex beads; thus, these two isolates expressed both a P-specific adhesin and an X adhesin and were the only two strains on which typical long fimbriae associated with MRHA could be detected by electron microscopy. All other strains expressing an X adhesin clearly exhibited the presence of organized material surrounding the bacteria, which for some of the strains (A2, A22, and A30) appeared as thin and "fuzzy" fimbrillae.

Structural comparison of the *afa* operon with the related chromosomal DNA sequences identified in the *E. coli* isolates. Total chromosomal DNA was extracted from the cells of the 15 *E. coli* isolates selected by colony hybridization, digested with restriction endonuclease *Hin*dIII (data not shown), *Pst*I (Fig. 2), or *Sma*I (Fig. 3), and hybridized with ^{32}P-labeled DNA restriction fragments from plasmid pILL03 or pILL14. Probe A consisted of the 11-kb *Hin*dIII fragment extending beyond the right boundary of the *afa* operon in pILL03 (Fig. 1); probe B consisted of the *Sma*I$_1$-*Sma*I$_2$ plus the *Pst*I$_3$-*Pst*I$_4$ pILL14 internal restriction fragments (Fig. 1); probe C consisted of the *Sma*I$_1$-*Bam*HI$_1$ pILL14 restriction fragment containing the last 83 C-terminal residues of the AFA-I adhesin polypeptide and the left boundary of the *afa* operon, as demonstrated by the nucleotide sequence previously published (18).

By using the A and B probes, it was apparent that the DNA homology of the clinical isolates detected by colony hybridization was due to the presence of genes identical or closely related to the *afaB*, *afaC*, and *afaD* genes. Thus, genomic DNA digested with *Pst*I (Fig. 2) gave rise to restriction fragments with the same electrophoretic mobility as those identified in pILL14 (i.e., 2.6, 1.1, and 0.4 kb). In contrast, the *Sma*I patterns of the strains revealed with the B probe (Fig. 3) showed that only three of the strains (A43, 6584, and 4006a) had the same 3-kb *Sma*I fragment present in both KS52 (Fig. 3) and pILL14 (Fig. 1), which extends down to the left end of the *afa* operon in pILL14. The other strains lacked this specific fragment, and the B probe hybridized to fragments with different electrophoretic mobility. These results indicate that the DNA sequences located on the left side of the *Pst*I$_1$ site are not conserved in the same arrangement as the one found in pILL14, which was cloned from the original strain KS52.

The same Southern blots of *Sma*I restriction endonuclease digests of total DNA were hybridized with the C probe (Fig. 3, Table 2), no homology was found between any of the strains lacking the specific 3-kb *Sma*I fragment (Fig. 3) and an *afa* DNA fragment extending from the *Bam*HI site located in the *afaE* gene and the *Sma*I$_1$ site. These data show that three strains (A43, 6584, and 4006a) had DNA sequences closely related to those of KS52, i.e., the entire KS52 6.7-kb DNA segment required for expression of the hemagglutination and adhesion properties. As there were no obvious modifications at the DNA level, those strains should express the products of the *afaA*, *afaB*, *afaC*, *afaD*, and *afaE* genes and therefore would be expected to produce the AFA-I adhesin. In contrast, all other strains lacked the specific region encoding the AFA-I adhesin (*afaE*).

These hybridization experiments also showed the following additional features. (i) The closely related strains KS52, 4006a, 6584, and A43 shared more DNA sequences than the

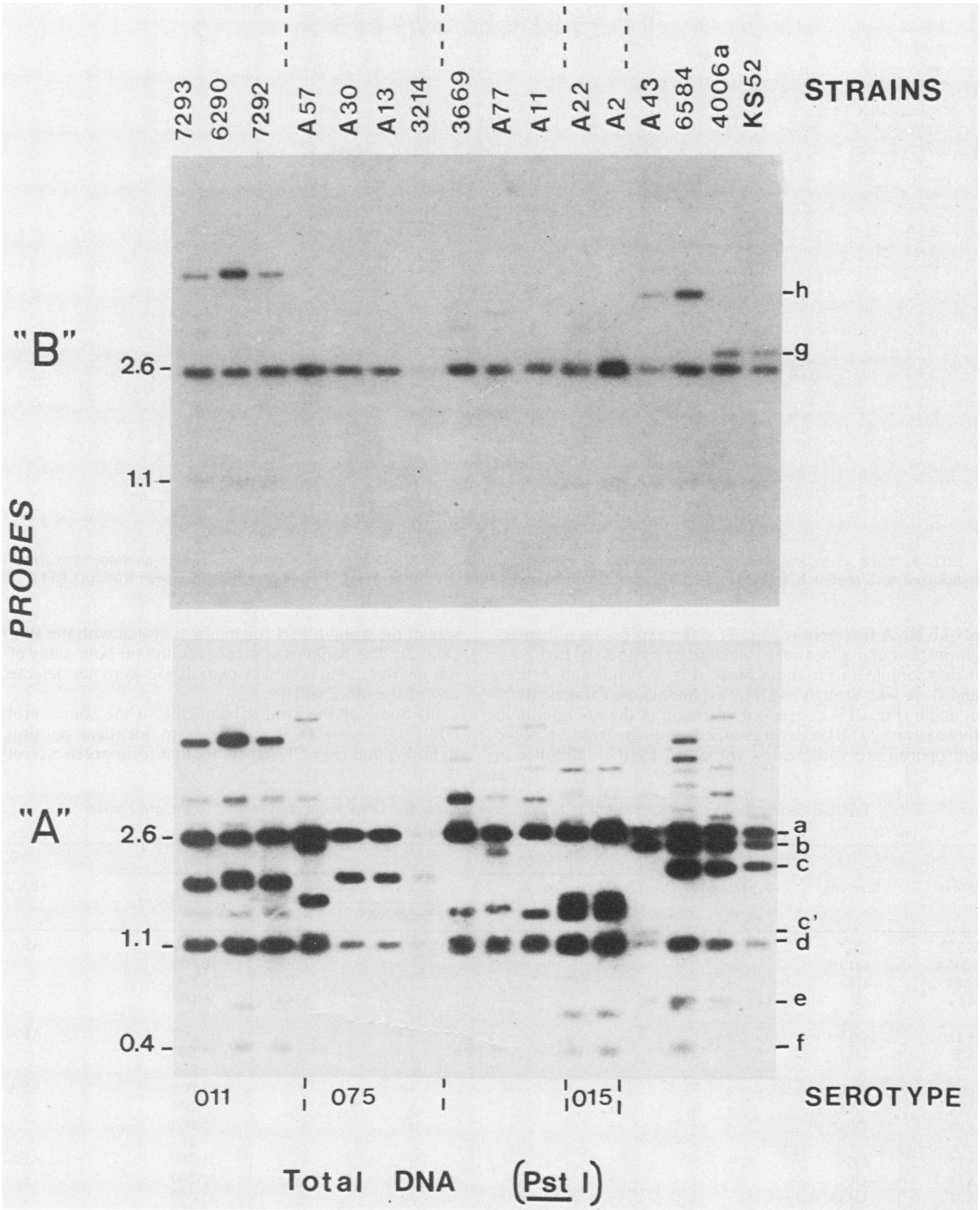

FIG. 2. Search for homology between the pILL14 hybrid plasmid and total DNA from *E. coli* isolates detected by autoradiography. Total DNA from AFA-related clinical isolates was digested with the restriction endonuclease *Pst*I, and the resulting fragments were separated by electrophoresis through a 0.7% agarose gel, transferred to nitrocellulose, and hybridized with the in vitro ^{32}P-labeled nick-translated probes A and B described in Fig. 1. The values correspond to the size (in kilobases) of the *Pst*I DNA fragments generated from pILL14. a to h, *Pst*I fragments corresponding to the conserved DNA region among *afa-1*-expressing strains.

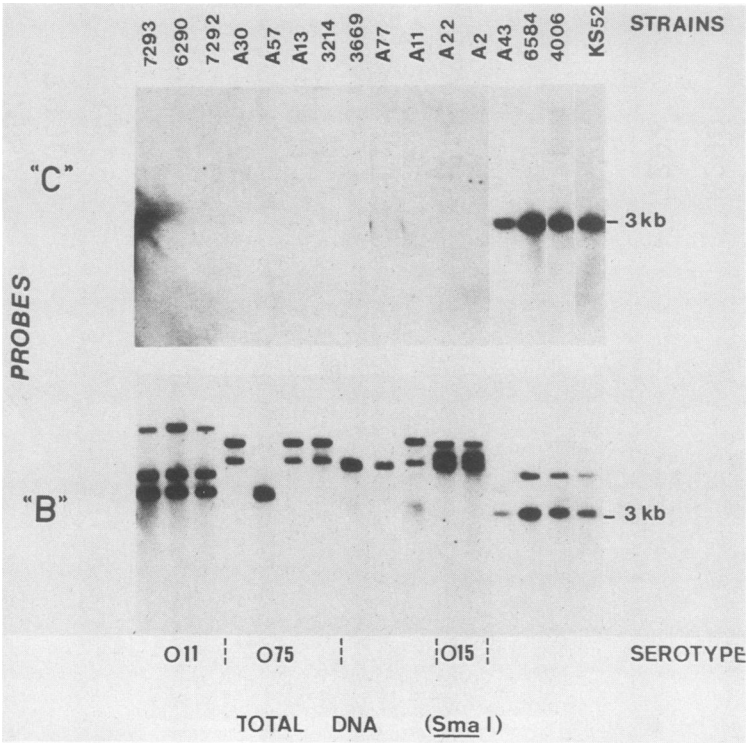

FIG. 3. Same as indicated in the legend to Fig. 2, except that total DNA was digested with the restriction endonuclease *Sma*I and hybridized with probes B and C (Fig. 1). The values correspond to the size of the *Sma*I internal fragment generated from pILL14.

6.7-kb DNA fragment required for the expression of hemagglutination and adhesion as evidenced by the six *Pst*I fragments homologous to the A probe (Fig. 2, bands a, b, c, d, e, and f). A 5-kb stretch of DNA expanding over fragments b, c, and e (Fig. 2) is conserved upstream of the *afa* operon in these strains. The bordering sequences downstream from the *afa* operon are variable, as witnessed by the difference in size of the g and h *Pst*I fragments revealed with the B probe (Fig. 2). The flanking sequences found on both sides of the *afa* operon in the original isolate KS52 were not detected in any of the other isolates.

(ii) Some of the strains belonging to the same serotype (O11, O15, and O75) gave rise to identical patterns of hybridization (Fig. 2), indicating that for a precise serotype

TABLE 2. Proposed classification of the *E. coli* strains harboring DNA sequences related to the *afa* operon based on hybridization and Western blots

Strain	Serotype	X phenotype	Hybridization		Western blots			AFA group
			B probe	C probe	Cross-reactivity		Mol wt	
					Denatured	Native		
KS52	O2	+	+	+	+++	+++	16,000	AFA-I
4006A	O19	+	+	+	+++	+++	16,000	
6584	O4	+	+	+	+++	+++	16,000	
A43	O1	+	+	+	+++	+++	16,000	
A2	O15	+	+	−	++	−	15,000	AFA-II
A22	O15	+	+	−	++	−	15,000	
3214	O75	+	+	−	+	−	15,000	AFA-III
A13	O75	+	+	−	+	−	15,000	
A30	O75	+	+	−	+	−	15,000	
A57	O75	−	+	−	+	−	15,500	AFA-IV
A11	O6	+	+	−	±	−	15,500	
A77	O133	−	+	−	±	−	15,500	
3669	O2	+	+	−	±	−	15,500	
7292	O11	+	+	−	±	−	16,000 + 15,500	
6290	O11	+	+	−	±	−	16,000 + 15,500	
7293	O11	+	+	−	±	−	16,000 + 15,500	

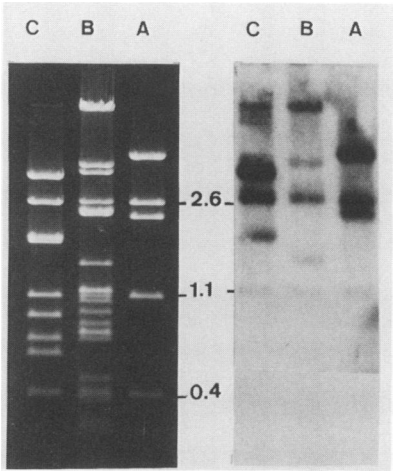

FIG. 4. Comparison of hybrid plasmids encoding AFA-related operons. Recombinant plasmids pILL14 (A), pILL72 (B), and pILL61 (C) were constructed from the total DNA of strains KS52, A22, and A30, respectively. Following digestion with *Pst*I (left panel), they all exhibited the characteristic 2.6-, 1.1-, and 0.4-kb *Pst*I fragments as demonstrated by Southern hybridization with hybrid plasmid pILL14 used as the probe (right panel).

the bordering sequences were conserved on both sides of the the *afa*-related sequences.

(iii) Due to the fact that only one *Sma*I site was present in the DNA sequence conserved in all the 15 strains (namely, the $PstI_1$-$PstI_4$ DNA fragment), we could expect that only two fragments of a *Sma*I digest of chromosomal DNA from these strains would hybridize to the B probe. This expectation was fulfilled with the majority of the strains. However, the strains belonging to serotypes O11 and O15 contained DNA sequences in which three *Sma*I fragments strongly hybridized to the B probe (Fig. 4). This suggests that two distinct gene clusters related to the *afa* operon are present in these strains. Another exception was found with strains A57 and A77, which do not express the X-adhesin phenotype (Table 1) and exhibit only one *Sma*I-hybridizing fragment. This suggests that in these latter strains the *afa* operon has undergone a deletion event leading to extinction of the X-adhesin phenotype.

Cloning of related *afa* operons from *E. coli* isolates lacking the AFA-I adhesin gene. Total DNA from *E. coli* A30 was partially digested with the endonuclease *Sau*3A to generate fragments ranging from 4 to 20 kb. They were sized on sucrose gradients (10 to 40%); fractions containing DNA fragments ranging from 7 to 13 kb long were pooled. The fragments were treated with alkaline phosphatase to prevent rearrangement of the original DNA sequences, and 1 μg of DNA was ligated to pBR322 linearized with *Bam*HI (75 ng). A total of 2,000 ampicillin-resistant transformants were screened by colony hybridization for the detection of A30 DNA sequences homologous to the A probe. The positive clones were tested for tetracycline sensitivity and MRHA phenotype. Of eight giving rise to a positive hybridization signal, one clone was selected which expressed the MRHA phenotype, adhered to uroepithelial cells, and contained a hybrid plasmid designated pILL61 (13.9 kb in size). Restriction pattern analysis of plasmid pILL61 demonstrated the presence of the three *afa*-internal *Pst*I fragments previously characterized for pILL14 (i.e., 2.6, 1.1, and 0.4 kb) (Fig. 4). On the other hand, pILL61 lacked the specific 3-kb *Sma*I fragment found with pILL14 (not shown).

Independently, bacteriophage lambda particles carrying recombinant cosmid molecules with fragments of A22 total DNA were prepared and used to transduce *E. coli* K-12 HB101. Of 400 colonies screened for their ability to agglutinate fresh human erythrocytes in the presence of D-mannose or digalactoside, three transformants harbored recombinant plasmids which generated the 2.6-, 1.1-, and 0.4-kb *Pst*I fragments (Fig. 4, pILL72) previously visualized when hybridizing the total DNA of strain A22.

DNA hybridization was performed between the C probe (containing part of the *afaE* structural gene) and DNA fragments generated from plasmids pILL61 and pILL72. None of these fragments shared DNA sequences with the *afaE* gene.

Occurrence of the AFA-I hemagglutinin or related hemagglutinin among the X^+ strains. Whole-cell protein extracts from the 15 AFA-related strains were subjected to SDS-PAGE, and antisera raised against the AFA-I protein were used in immunoblotting experiments to determine the frequency of the AFA-I adhesin.

Strains KS52, 4006a, 6584, and A43, which have a common *afaE* DNA sequence, as determined by hybridization experiments, produced a 16,000-M_r (16K) polypeptide which strongly cross-reacted with antisera raised against the denatured (Fig. 5-I) or native (Fig. 5-III) AFA-I polypeptide. These were the only strains for which cross-reactivity with antisera generated against the native polypeptide could be detected.

The antiserum raised against the denatured AFA-I protein recognized a 15K polypeptide expressed in strains A2 and A22 (belonging to serotype O15) as well as in strains 3214, A13, and A30 (serotype O75). In addition, a weak cross-reaction signal could be detected with polypeptides of 15.5K expressed by strains A11, A77, and 3669 and a strong signal with a 15.5K polypeptide expressed by strain A57.

The strains belonging to serotype O11 encoded two polypeptides (15.5K and 16K) which were both weakly recognized by the anti-AFA-I adhesin rabbit serum. All of the above results are summarized in Table 2.

DISCUSSION

Uropathogenic strains of *E. coli* usually produce surface adhesins that agglutinate erythrocytes and bind epithelial cells. The majority of pyelonephretic *E. coli* strains are associated with expression of the P blood group antigen-binding adhesin. However, some of the uropathogenic strains agglutinate erythrocytes lacking the P blood group antigen but still adhere to uroepithelial cells, suggesting the presence of additional, P-independent adhesins among the uropathogenic *E. coli*. The pyelonephritic *E. coli* strain KS52 was one of the isolates which was unable to bind to the digalactoside receptor but adhered to squamous and transitional uroepithelial cells as well as HeLa and Hep-2 epithelial cells. The genetic determinant responsible for the expression of this so-called X adhesin was cloned, and the operon was designated *afa-1* Transformation of nonadherent recipient strains with recombinant plasmids carrying the *afa-1* operon confers binding specificities and biochemical properties different from those observed with strains expressing the common mannose-binding adhesin (*pil* operon) or the Gal-Gal globoside-binding adhesin (*pap* operon), but also from those expressed by the minor group of uropathogenic *E. coli* exhibiting sialic acid as well as M and *N*-acetyl-D-glucosamine specificities.

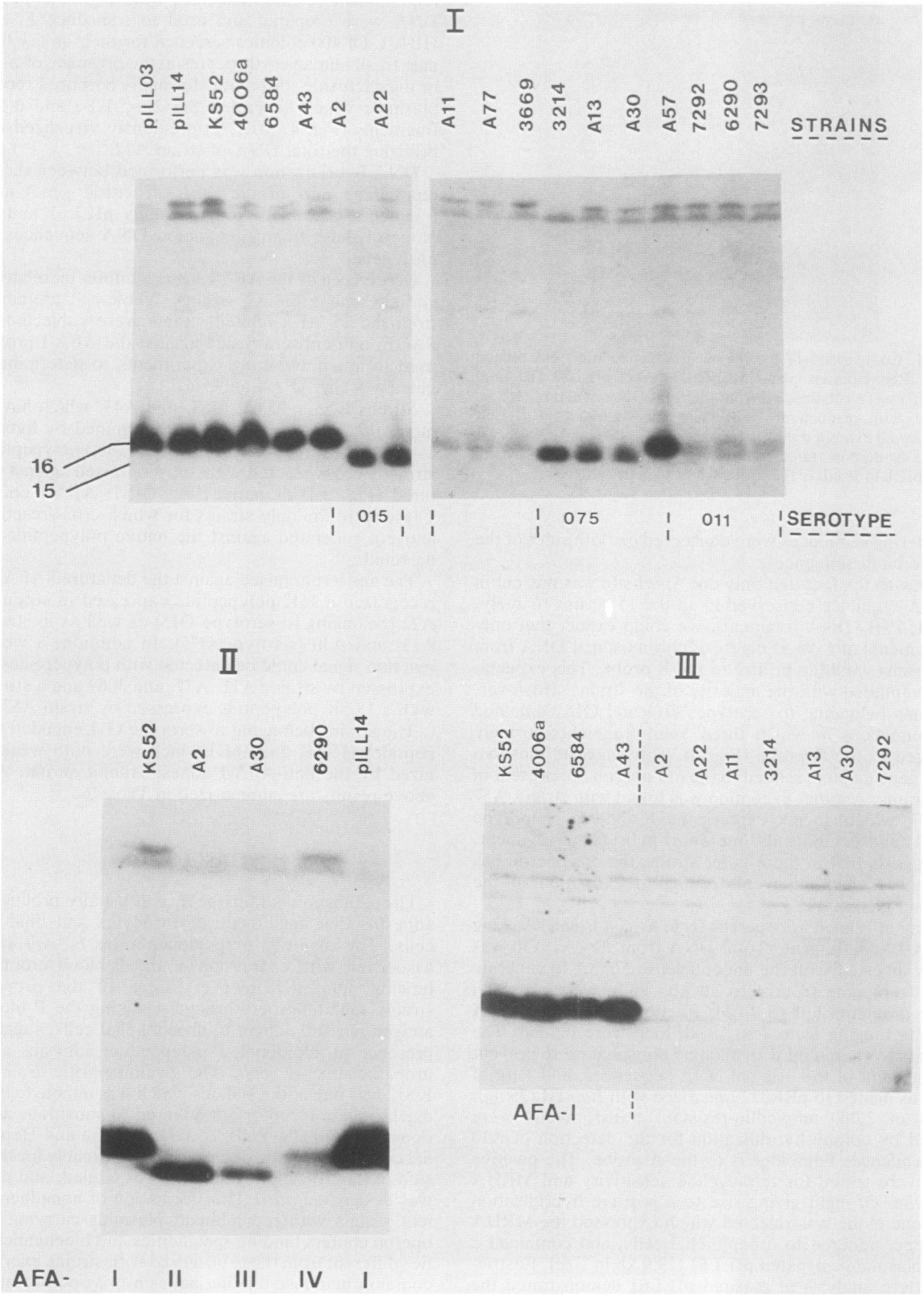

FIG. 5. Immunoblots of the SDS-PAGE of bacterial lysates incubated with rabbit antiserum raised against the denatured AFA-I protein (I and II) or the native AFA-I protein (III), followed by incubation with ^{125}I-protein A (0.5 μCi at 40 mCi/mg). Numbers indicate the molecular weights (in thousands) of the proteins cross-reacting with the AFA-I antisera.

In this study, we investigated the distribution of genetic material homologous to the *afa* operon characterized from the pyelonephritic isolate KS52, among UTI strains. We previously showed (17) that the *afa* sequences did not exhibit homology at the DNA level with cloned sequences of the *pil* operon (23, 26), *pap* operon (19), *sfa* operon (29) (unpublished results), or the genomic DNA of various laboratory *E. coli* strains. Hence, we could use DNA fragments internal to the *afa* operon as probes for the detection of *afa*-related sequences, and of 100 UTI isolates (including pyelonephritis, cystitis, and asymptomatic), 13 isolates were shown to contain DNA sequences related to the *afa* operon, a frequency significantly higher than the one observed with the population of fecal isolates (5%). Although the number of pyelonephritic isolates was low (15 isolates) compared with that of cystitis isolates (79 isolates), the distribution of the *afa*-related sequences did not seem to be associated more frequently with one or the other category. Strains containing sequences related to the *afa* operon belong to a large range of serotypes; no correlation could be established between the serogroup and the presence of an *afa* operon. In contrast, it was a common characteristic for all the *afa*-related isolates to be nonhemolytic, although a significant proportion of UTI isolates are commonly described as hemolysin producers (15, 20).

The use of *afa-1* operon internal fragments as probes (probe B) for the detection of AFA-encoding isolates was very selective. All the positive signals obtained by colony hybridization correspond to the presence of an *afa* operon in the genome of the isolates. Probe B potentially represents a useful tool for the detection and identification of AFA-expressing strains as long as that receptor molecule remains unknown. Whereas all the selected isolates harbored a highly conserved 4-kb DNA segment internal to the *afa-1* operon, only 4 of 15 isolates hybridized with a probe corresponding to the structural gene coding for the adhesin AFA-I. Nine of the *afa*-related isolates expressing an X adhesin lacked the *afaE* structural gene. Thus, it was not clear whether the DNA sequences related to the *afa* operon in those strains were responsible for expression of the X adhesin. We cloned from two of those X-adhesin-encoding strains (A30 and A22) the genetic determinants responsible for the X phenotype. In each case, the cloned DNA sequences contained a significant part of the *afa* operon, lacked the *afaE* gene, but still expressed an X adhesin. It was therefore clear that those isolates, and by extension the X-adhesin-expressing strains selected by colony hybridization, harbor a functional *afa* operon but contain a structural adhesin-encoding gene unrelated at the DNA level to the *afaE* gene.

The variability of the adhesins synthesized by the related *afa* operons was confirmed by Western blot analyses. Antibodies raised against the native AFA-I adhesin did not cross-react with any of the polypeptide synthesized by cells lacking the *afaE* gene. However, cross-reactivities were detected when antisera raised against the denatured AFA-I protein were used for immunologic analysis. This finding suggests that these cross-reacting adhesin peptides may represent serological variants of the AFA adhesin in which nonimmunogenic regions have been conserved when immunologic regions are variable. These findings are reminiscent of the antigenic diversity demonstrated for the P-fimbriae subunits (28) and K88 fimbrillae (11), whereas they contrast with the antigenic homogeneity of the mannose-sensitive (2) and S fimbriae (29). Subsequent molecular analysis of the gene encoding the AFA adhesin in non-*afa-1* operon-harboring strains, including DNA sequencing, should allow us to confirm the existence of these different domains and presumably to identify the receptor-binding domain of the protein.

Based on the hybridization patterns obtained with the different probes and the Western blot analyses, we were able to define different related *afa* operon families (Table 2). Each family included serologically homologous AFA isolates. Whereas we can rely on the AFA-I group as established in this work, the homogeneity of the AFA-II, AFA-III, and AFA-IV groups remains to be confirmed by further DNA and serological analyses. In the same way, we will have to establish by direct molecular cloning whether the two copies of the *afa* determinant detected in the chromosome of the isolates belonging to serotypes O11 and O15 are functional and whether they express AFA adhesins differing in their antigenicity.

Väisänen-Rhen recently published the characterization of an X-type fimbrialike hemagglutinin of *E. coli* O75 strains, termed O75-X hemagglutinin (36); no genetic cloning of the adhesin has been published yet to allow comparison of the O75-X determinant with the AFA determinant. The phenotypical characteristics of the O75-X hemagglutinin are very similar to those that we identified as AFA adhesin; however, the amino acid composition of the O75-X hemagglutinin does not correspond to our published composition (18) of the AFA-I adhesin. Our random screening of UTI isolates for *afa*-related sequences identified 4 of 15 isolates as belonging to the O75 serotype. Their hybridization patterns and Western blot responses suggest that these O75 strains form a clonal group of strains. It is possible, therefore, that the O75 hemagglutinin and the AFA-III adhesin are in fact the same protein. The DNA sequence of the AFA-III-encoding gene from the pILL61 recombinant plasmid will allow us to test this hypothesis.

Similarly, Orskov et al. (27), Hinson et al. (9), and Williams et al. (38) described adhesin proteins from *E. coli* strains isolated from infantile enteritis with the same general properties as the AFA-I adhesin. Comparison at the DNA level and protein level of those strains with the AFA-encoding strains suggests that the Z1 antigen described by Orskov et al. (27) and the adhesin from strain 469-3 (9) are expressed by *afa-1*-related operons in those strains and correspond to the AFA-I adhesin (M. Archambaud, V. Ouin, P. Courcoux, and A. Labigne-Roussel, manuscript in preparation). The *afa* operon distribution thus does not appear to be restricted to UTI *E. coli* strains, and larger studies have to be carried out to establish the distribution of the *afa* operons in pathogenic *E. coli*, including enteropathogenic and enterotoxigenic strains and *E. coli* causing extraintestinal infections.

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LITERATURE CITED

1. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *E. coli*. J. Mol. Biol. **41**:459–472.
2. **Buchanan, K., S. Falkow, R. A. Hull, and S. Hull.** 1985. Frequency among *Enterobacteriaceae* of the DNA sequences encoding type 1 pili. J. Bacteriol. **162**:799–803.
3. **Cohen, M. L., and S. Falkow.** 1981. Protein antigens from

Staphylococcus aureus strains associated with toxic-shock syndrome. Science **211**:842–844.
4. **Collins, J.** 1979. *Escherichia coli* plasmids packageable in vitro in bacteriophage particles. Methods Enzymol. **68**:309–326.
5. **Denhardt, D. T.** 1966. A membrane filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
6. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of *Enterobacteriaceae*, p. 185–217. *In* E. H. Beachey (ed.), Receptors and recognition, series B, vol. 6: bacterial adherence. Chapman and Hall, Ltd., London.
7. **Grunstein, M., and D. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA **72**:3961–3965.
8. **Hagberg, L., U. Jodal, T. K. Korhonen, G. Lidin-Johnson, U. Linberg, and C. Svanborg-Eden.** 1981. Adhesion, hemagglutination, and virulence of *Escherichia coli* causing urinary tract infections. Infect. Immun. **31**:564–570.
9. **Hinson, G., S. Knutton, M. K.-L. Lam-Po-Tang, A. S. McNeish, and P. H. Williams.** 1987. Adherence to human colonocytes of an *Escherichia coli* strain isolated from severe infantile enteritis: molecular and ultrastructural studies of a fibrillar adhesin. Infect. Immun. **55**:393–402.
10. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
11. **Jacobs, A. A. C., J. Venema, R. Leeven, H. van Pelt-Heerschap, and F. K. de Graaf.** 1987. Inhibition of adhesive activity of K88 fibrillae by peptides derived from the K88 adhesin. J. Bacteriol. **169**:735–741.
12. **Kallenius, G., R. Mollby, S. B. Svenson, J. Windberg, A. Lundblad, S. Svenson, and B. Cedergren.** 1980. The p^k antigen as receptor for the haemagglutination of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. **7**:297–302.
13. **Kallenius, G., S. B. Svenson, R. Mollby, B. Cedergren, and H. Hultberg.** 1981. Structure of carbohydrate part of receptor on human uroepithelial cells for pyelonephritogenic *Escherichia coli*. Lancet **ii**:604–606.
14. **Kallenius, G., S. B. Svenson, R. Mollby, T. Korhonen, J. Winberg, B. Cedergren, I. Helin, and H. Hulterg.** 1982. Carbohydrate receptor structures recognized by uropathogenic *E. coli*. Scand. J. Infect. Dis. Suppl. **33**:52–60.
15. **Knapp, S., J. Hacker, I. Then, D. Muller, and W. Goebel.** 1984. Multiple copies of hemolysin genes and associated sequences in the chromosome of uropathogenic *Escherichia coli* strains. J. Bacteriol. **159**:1027–1033.
16. **Korhonen, T. K., V. Vaisanen-Rhen, M. Rhen, A. Pere, J. Parkkinen, and J. Finne.** 1984. *Escherichia coli* fimbriae recognizing sialyl galactosides. J. Bacteriol. **159**:762–766.
17. **Labigne-Roussel, A. F., D. Lark, G. S. Schoolnik, and S. Falkow.** 1984. Cloning and expression of an afimbrial adhesin (AFA-I) responsible for P blood group-independent, mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli* strain. Infect. Immun. **46**:251–259.
18. **Labigne-Roussel, A. F., M. A. Schmidt, W. Walz, and S. Falkow.** 1985. Genetic organization of the *afa* operon and nucleotide sequence from a uropathogenic *Escherichia coli* gene encoding an afimbrial adhesin (AFA-I). J. Bacteriol. **162**:1285–1292.
19. **Lindberg, F. P., B. Lund, and S. Normark.** 1984. Genes of pyelonephritogenic *E. coli* required for digalactoside specific agglutination of human cells. EMBO J. **3**:1167–1173.
20. **Low, D., V. David, D. Lark, G. Schoolnik, and S. Falkow.** 1984. Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotype O4 and O6 isolates from urinary tract infections. Infect. Immun. **43**:353–358.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1983. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward of phage lambda. Proc. Natl. Acad. Sci. USA **72**:1184–1188.
23. **Maurer, L., and P. E. Orndorff.** 1987. Identification and characterization of genes determining receptor binding and pilus length of *Escherichia coli* type 1 pili. J. Bacteriol. **169**:640–645.
24. **Norgren, M., S. Normak, D. Lark, P. O'Hanley, G. Schoolnik, S. Falkow, C. Svanborg-Eden, M. Baga, and B. Uhlin.** 1984. Mutations in *E. coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation. EMBO J. **3**:1159–1169.
25. **Normark, S., D. Lark, R. Hull, M. Norgren, M. Baga, P. O'Hanley, G. Schoolnik, and S. Falkow.** 1983. Genetics of digalactoside-binding adhesin from a uropathogenic *Escherichia coli* strain. Infect. Immun. **41**:942–949.
26. **Orndoff, P. E., and S. Falkow.** 1984. Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. J. Bacteriol. **159**:736–744.
27. **Orskov, I., A. Birch-Andersen, J. P. Duguid, J. Stenderup, and F. Orskov.** 1985. An adhesive protein capsule of *Escherichia coli*. Infect. Immun. **47**:191–200.
28. **Orskov, I., and F. Orskov.** 1983. Serology of *Escherichia coli* fimbriae. Prog. Allergy **33**:80–105.
29. **Ott, M., J. Hacker, T. Schmoll, T. Jarchau, T. K. Korhonen, and W. Goebel.** 1986. Analysis of the genetic determinants coding for the S-fimbrial adhesin (*sfa*) in different *Escherichia coli* strains causing meningitis or urinary tract infections. Infect. Immun. **54**:646–653.
30. **Parkkinen, J., J. Finne, M. Achtman, V. Vaisanen, and T. K. Korhonen.** 1983. *Escherichia coli* strains binding neuraminyl 2-3 galactosides. Biochem. Biophys. Res. Commun. **111**:456–461.
31. **Rhen, M., P. Klemm, and T. K. Korhonen.** 1986. Identification of two new hemagglutinins of *Escherichia coli*, *N*-acetyl-D-glucosamine-specific fimbriae and a blood group M-specific agglutinin, by cloning the corresponding genes in *Escherichia coli* K-12. J. Bacteriol. **168**:1234–1242.
32. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
33. **Svanborg-Eden, C., L. A. Hanson, U. Jodal, U. Lindberg, and A. Sohl Akerlund.** 1976. Variable adhesion to normal urinary tract infection. Lancet **ii**:490–492.
34. **Vaisanen, V., T. K. Korhonen, M. Jokinen, C. G. C. Ahmberg, and C. Ehnholm.** 1982. Blood group M specific haemagglutinin in pyelonephritogenic *Escherichia coli*. Lancet **i**:1192.
35. **Vaisanen, V., L. G. Tallgren, P. H. Makela, G. Kallenius, H. Hultberg, J. Elo, A. Siitonenm, C. Svanborg-Eden, S. B. Svenson, and T. Korhonen.** 1981. Mannose-resistant haemagglutination and P antigen recognition are characteristic of *Escherichia coli* causing primary pyelonepritis. Lancet **ii**:1366–1369.
36. **Vaisanen-Rhen, V.** 1984. Fimbria-like hemagglutinin of *Escherichia coli* O75 strains. Infect. Immun. **46**:401–407.
37. **Walz, W., M. A. Schmidt, A. F. Labigne-Roussel, S. Falkow, and G. Schoolnik.** 1985. AFA-I, a cloned afimbrial X-adhesin from a human pyelonephritic *Escherichia coli* strain. Purification, and chemical, functional and serologic characterization. Eur. J. Biochem. **152**:315–321.
38. **Williams, P. H., S. Knutton, M. G. M. Brown, D. C. A. Candy, and A. S. McNeish.** 1984. Characterization of nonfimbrial mannose-resistant protein hemagglutinins of two *Escherichia coli* strains isolated from infants with enteritis. Infect. Immun. **44**:592–598.