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Analysis of the Genetic Determinants Coding for the S-Fimbrial Adhesin (*sfa*) in Different *Escherichia coli* Strains Causing Meningitis or Urinary Tract Infections

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Recently we have described the molecular cloning of the genetic determinant coding for the S-fimbrial adhesin (Sfa), a sialic acid-recognizing pilus frequently found among extraintestinal *Escherichia coli* isolates. Fimbriae from the resulting Sfa$^+$ *E. coli* K-12 clone were isolated, and an Sfa-specific antiserum was prepared. Western blots indicate that S fimbriae isolated from different uropathogenic and meningitis-associated *E. coli* strains, including O83:K1 isolates, were serologically related. The Sfa-specific antibodies did not cross-react with P fimbriae, but did cross-react with F1C fimbriae. Furthermore the *sfa*$^+$ recombinant DNAs and some cloned *sfa*-flanking regions were used as probes in Southern experiments. Chromosomal DNAs isolated from O18:K1 and O83:K1 meningitis strains with and without S fimbriae and from uropathogenic O6:K$^+$ strains were hybridized against these *sfa*-specific probes. Only one copy of the *sfa* determinant was identified on the chromosome of these strains. No *sfa*-specific sequences were observed on the chromosome of *E. coli* K-12 strains and an O7:K1 isolate. With the exception of small alterations in the *sfa*-coding region the genetic determinants for S fimbriae were identical in uropathogenic O6:K$^+$ and meningitis O18:K1 and O83:K1 strains. The *sfa* determinant was also detected on the chromosome of K1 isolates with an Sfa-negative phenotype, and specific cross-hybridization signals were visible after blotting against F1C-specific DNA. In addition homology among the different strains was observed in the *sfa*-flanking regions.

Escherichia coli isolates can cause several extraintestinal infections. Urinary tract infections and cases of neonatal sepsis and meningitis are frequently found among the human population, and the former is one of the main infectious diseases in general (23, 25; M. Achtman and G. Pluschke, Annu. Rev. Microbiol., in press). Certain virulence properties, i.e., specific O and K antigens, serum resistance, specific iron uptake systems, and synthesis of hemolysin and of different adhesins, are involved in the pathogenesis of these diseases (12, 29, 38).

The adhesins (mostly associated with fimbriae or pili) which are responsible for the specific attachment of bacteria to eucaryotic cells can be divided into different groups by their affinity to specific receptor structures like α-D-Gal-(1-4)-β-D-Gal (P adhesins; 16), α-D-mannosides (MS adhesins [30]), sialic acids (S adhesins [20]), or glycophorin A (M adhesins [37]). Certain fimbriae such as those of serotype F1C are devoid of demonstrable receptor specificity (32). The P adhesins are most frequently found among strains from urinary tract infections (38), whereas the S and MS adhesins can be detected on *E. coli* strains isolated from different sources. The S-fimbrial adhesins (Sfa), however, are most often found among meningitis- and sepsis-associated isolates (11, 21).

Recently we have cloned the genetic determinant coding for the S-fimbrial adhesin (*sfa*) from the chromosome of the uropathogenic O6:K15:H31 strain 536 (10). The *sfa* determinant (previously termed X determinant) covers a stretch of 6.5 kilobases (kb) of DNA and codes for at least seven *sfa*-specific gene products, including the S-fimbrial protein subunits of 16.5 kilodaltons in size (10; Hacker and Düvel, unpublished results). S fimbriae are subject to phase variation in vivo (B. Nowicki, J. Vuopio-Varkila, P. Viljanen, T. K. Korhonen, and P. H. Mäkelä, Microb. Pathogenesis, in press), and their expression is favored inside the infected host. Thus they appear to play a role in the pathogenesis of systemic infections in the mouse (Nowicki et al., in press) and of urinary tract infections in the rat (J. Hacker, T. Jarchau, S. Knapp, R. Marre, G. Schmidt, T. Schmoll, and W. Goebel, *in* D. Lark, S. Normark, H. Wolf-Watz, and B. E. Uhlin, ed., *Molecular Biology of Microbial Pathogenicity*, in press). It has been demonstrated that the Sfa-negative phenotype of a 536 mutant strain is due to a block in transcription of the *sfa* genes (17) similar to what has also been shown for the MS-fimbria determinant (7).

Here we present evidence that the S fimbriae from O6:K$^+$ urinary tract infection and K1 meningitis *E. coli* strains are serologically related. Their corresponding *sfa* determinants consist of DNA sequences which are highly conserved in their coding and flanking regions. In addition we demonstrate that S-negative variants of serotypes which normally exhibit the Sfa still carry one copy of the *sfa* determinant on the chromosome.

MATERIALS AND METHODS

Media, chemicals, and enzymes. Bacteria were grown in enriched nutrient broth or in alkaline broth extract. Radiochemicals were purchased from New England Nuclear Corp., Boston, Mass.; antibiotics were a gift from Bayer, Leverkusen, Federal Republic of Germany. All other chemicals were obtained from E. Merck AG, Darmstadt, Federal Republic of Germany. Restriction enzymes and T4 ligase were purchased from Bio-Rad Laboratories, Richmond,

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TABLE 1. *E. coli* wild-type strains tested

Strain	Serotype/outer membrane protein pattern[a]	Source[b]	Hemolysin production	Adhesin	Reference
536	O6:K15:H31	UTI	+	Sfa, F1[c]	9
RS218	O18:K1:H7/6	NBM	+	Sfa, F1	21
RS226	O18:K1:H7/6	FEC	+	Sfa, F1	21
IH3034	O18:K1:H7/9	NBM	−	Sfa, F1	21
IH3036	O18:K1:H7/9	NBM	−	F1	21
IH3095	O6:K$^+$:H$^+$	UTI	−	Sfa, F1	38
A21	O7:K1:H$^+$/3	NBM	−	P(F?), F1	Achtman et al.[d]
A1396	O83:K1:H?/32	NBM	−		Achtman et al.
A1520	O83:K1:H?/32	NBM	−		Achtman et al.
A1564	O83:K1:H?/32	NBM	−		Achtman et al.
A1386	O83:K1:H?/32	NBM	−		Achtman et al.
A1563	O83:K1:H?/32	NBM	−		Achtman et al.
A1566	O83:K1:H?/32	NBM	−		Achtman et al.
A1565	O83:K1:H?/32	NBM	−		Achtman et al.
A1522	O83:K1:H?/32	NBM	−		Achtman et al.
A1521	O83:K1:H?/32	NBM	−	Sfa	Achtman et al.
A1523	O83:K1:H?/32	NBM	−	Sfa	Achtman et al.
764	O18:K5:H$^-$/11	FEC	+	P(F8?), F1C	2
2980	O18:K5:H5/11	FEC	+	P(F8), F1C	41

[a] Serotype and outer membrane protein patterns were from references 21 and 41 and Achtman et al. (in press).
[b] UTI, Urinary tract infection; NBM, newborn meningitis; FEC, fecal isolate.
[c] F1 fimbriae represent mannose-sensitive hemagglutination adhesins; F1C fimbriae represent the pseudotype I fimbriae without any hemagglutination activity; F8 represents the P fimbriae of serogroup F8.
[d] Annu. Rev. Microbiol., in press.

Calif. DNA polymerase I was obtained from Boehringer, Mannheim, Federal Republic of Germany.

Bacteria and recombinant DNAs. The wild-type strains used are listed in Table 1. For transformation, the *E. coli* K-12 strains HB101 and 5K were used. The *sfa*-specific recombinant DNAs were derived from the *sfa*-coding region of the uropathogenic strain 536 (O6:K15:H31) and are listed in Table 2. These DNAs, used for the preparation of the *sfa*-specific probes, are additionally indicated in Fig. 1. The recombinant plasmid pANN921, which codes for the P fimbriae of serogroup F8, has been described elsewhere (J. Hacker, M. Ott, G. Schmidt, R. Hull, and W. Goebel, FEMS Microbiol. Lett., in press), and the F1C-specific recombinant DNA pPIL 110-54 (39) was kindly supported by Irma VanDie (Utrecht, The Netherlands).

Isolation of chromosomal and plasmid DNA. Chromosomal DNA was isolated as described earlier (18). Plasmid DNA from clones carrying recombinant DNA was screened by the cleared lysate procedure (3) and preparative DNA isolation was done as described previously (8). DNA fragments were isolated after agarose gel electrophoresis by electroelution (26).

Nick translation. Only defined DNA fragments eluted from agarose gels were used as DNA probes (Fig. 1). These DNA fragments were labeled by nick translation with a mixture of all four α-^{32}P-labeled deoxynucleoside triphosphates as described previously (31) and purified by ethanol precipitation.

Cleavage with restriction enzymes and electrophoresis of chromosomal DNA. The chromosomal DNA was treated with appropriate restriction enzymes, and the resulting fragments were separated by agarose gel electrophoresis with 0.7 to 1.0% gels as described previously (18).

Hybridization and autoradiography. The transfer of DNA fragments from agarose gels to nitrocellulose filters and the washing and autoradiography of the filters after hybridization were performed as described previously (34). The filters were hybridized in 50% formamide at 43°C for 3 days. Stringent conditions were used for the washing procedure.

Transformation. *E. coli* K-12 strains were transformed by the $CaCl_2$ method. Wild-type strains were transformed by a modified $CaCl_2$ procedure (22).

Hemolysin production. Erythrocyte lysis was detected on meat agar plates containing washed human erythrocytes and confirmed in a liquid assay (40).

Preparation of fimbriae. Fimbriae were isolated from wild-type strains or from *E. coli* K-12 bacteria harboring cloned fimbria determinants. S fimbriae used for the immunization of rabbits were prepared from an Sfa$^+$ *E. coli* K-12 clone which was negative for other fimbrial antigens, including F1C fimbriae. The bacteria used for the preparation of fimbriae were grown on plates (15).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fimbrial protein subunits. After disintegration of the fimbriae by boiling for 5 min in 10 mM Tris hydrochloride (pH 7.8) containing 4% sodium dodecyl sulfate, 0.01 ml of mercaptoethanol per ml, 0.2 ml of glycerol per ml, and 0.002% bromphenol blue, the fimbrial samples (15 μg of

TABLE 2. Recombinant DNAs

Recombinant plasmid	Characteristics	Reference
pANN801	Ap^r, *sfa*$^{+a}$ (cosmid)	10
pANN801-13	Ap^r, *sfa*$^+$ (coordinates 0 to 10.7 kb)[b]	10
pANN801-12	Tc^r, *sfa*-specific sequences (coordinates 0 to 6.1 kb)	10
pANN801-131	Cm^r, *sfa*-specific sequences (coordinates 6.1 to 7.7 kb)	This study
pANN801-15	Cm^r, *sfa*-specific sequences (coordinates 7.7 to 10.5 kb)	This study
pANN921	Ap^r, P(F8)	Hacker et al.[c]
pPIL110-54	Cm^r, F1C	39

[a] *sfa* refers to the sfa determinant, cloned from the chromosome of strain 536.
[b] The coordinates mark the cloned *sfa* specific insert DNA as indicated in Fig. 1.
[c] FEMS Microbiol. Lett., in press.

protein) were run on slab gels as described previously (15, 41).

Preparation of antisera. S-fimbria-specific antisera were prepared from rabbits with purified fimbrial protein eluted from a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel (15). The preimmune sera did not react with any fimbriae isolated.

Determination of adhesins. Mannose-resistant hemagglutination was determined after mixing the bacterial cells with human or bovine erythrocytes with and without 2% mannose. P-specific adhesion was estimated with P1 and p̄ erythrocytes (16). Mannose-sensitive hemagglutination was confirmed by agglutination of yeast cells (19). Sfa were determined with erythrocytes treated with neuraminidase (20). The presence of the Sfa was further confirmed by Western blots with antiserum against cloned and purified S fimbriae of strain 536. Western blotting was carried out by the method of Towbin et al. (35).

RESULTS

Estimation of fimbriae on different *E. coli* wild-type strains. Wild-type *E. coli* strains isolated from different sources were tested for the presence of different adhesins (Table 1). In agreement with previous results (11, 15, 21), O6:K$^+$ strains (536, IH3095) and O18:K1 meningitis isolates (RS218, RS226, IH3034) exhibited the Sfa and MS fimbriae. The O18:K5 isolates (764, 2980) carried P(F8) and F1C fimbriae. One meningitis strain of serotype O7:K1 (A21) produced P fimbriae. From 10 O83:K1 meningitis strains tested, 2 isolates (A1521, A1523) carried S fimbriae. These two O83:K1 Sfa$^+$ strains and one of their Sfa$^-$ variants (A1396) were further characterized.

Detection of the Sfa antigen on *E. coli* wild-type strains. In Western blot analysis of fimbriae isolated from the wild-type strains listed in Table 1, the *E. coli* K-12 isolate HB101 and the *E. coli* HB101 strain harboring recombinant DNAs were blotted against Sfa-specific antiserum isolated from rabbits which had been immunized with cloned purified S fimbriae of strain HB101 (pANN801-13). The cloned *sfa* determinant was derived from the urinary tract infection strain 536 (O6:K15:H31). The S fimbriae of the different strains reacted with the antiserum in a protein band of 16.5 to 17 kilodaltons (Fig. 2). Small differences in the molecular weights and in the amount of the Sfa subunits produced by the different strains were visible. Fimbria preparations of the Sfa$^-$ O83:K1

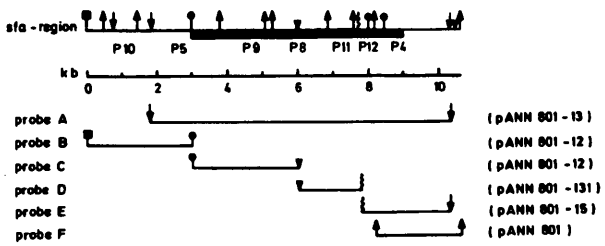

FIG. 1. Physical map of the S-fimbrial adhesin determinant (*sfa*) and *sfa* flanking regions of strain 536. The coordinates of the *sfa* determinant start from the left *Bam*HI site. The *Pst*I fragments are designated as P10 (left) to P14 (right). The thick bar represents the *sfa*-coding region as described elsewhere (10). The thin lines in the lower part represent the different probes, A through G, used for Southern hybridizations. The terms in the brackets represent the recombinant DNAs from which the probes have been isolated (see Table 2). Symbols: ■, *Bam*HI; ▼, *Eco*RV; ▲, *Pst*I; ●, *Sph*I; ▼, *Eco*RI; ⦚, *Cla*I.

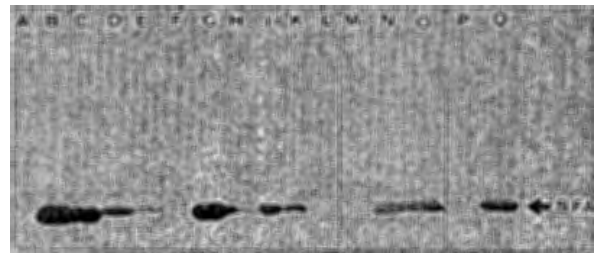

FIG. 2. Western blots of fimbrial protein preparations (nearly 30 μg of protein was used) from different strains probed against anti Sfa-antiserum. Lanes: A, HB101; B, HB101 (pANN801-13) (Sfa); C, 536; D, A1521; E, A1523; F, A1396; G, HB101 (pPIL110-54) (F1C); H, IH3095; I, 2980; K, 764; L, HB101 (pANN921) (F8); M, A21; N, RS218; O, RS226; P, IH3036; Q, IH3034.

(A1396) and O18:K1 (IH3036) strains (Fig. 2, lanes F and P) did not react with Sfa-specific antiserum, as was also the case with fimbriae from the O7:K1 strain A21.

Fimbriae prepared from the O18:K5 strains reacted with the Sfa-specific antiserum (Fig. 2, lanes I and K) in one band of 17 kilodaltons. To determine whether the F8 or the F1C fimbriae of these strains were responsible for the cross-reaction, *E. coli* K-12 strains which harbored the recombinant plasmids pANN921 and pPIL110-54 were used. The former plasmid codes for F8 fimbriae (Hacker et al., FEMS Microbiol. Lett., in press), and the latter codes for F1C fimbriae (39). The Sfa-specific antiserum reacted with the cloned F1C fimbriae (Fig. 2, lane G) but not with the F8 fimbriae (lane L). Thus it was obvious that the additional 17-kDa band of the O18:K5 strains resulted from a reaction of Sfa antibodies with F1C fimbriae (Fig. 2, lane G), a result which was expected from the molecular weights of the F8 (19.5-kDa) and the F1C (17-kDa) fimbrial subunits.

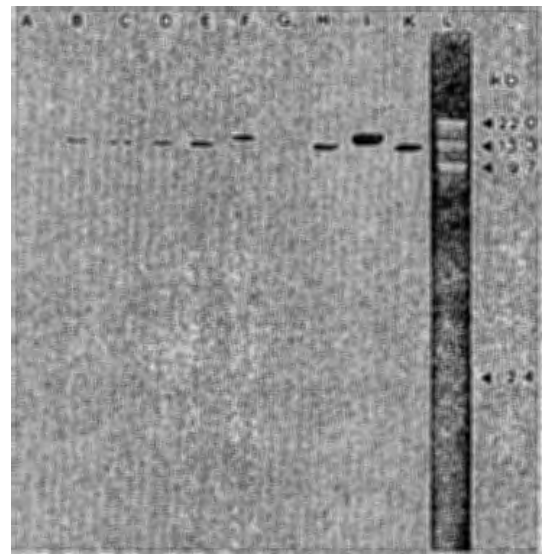

FIG. 3. Southern hybridization pattern of *Bam*HI-*Sal*I double-cleaved total DNA of *E. coli* strains HB101 (A); RS218 (B), RS226 (C), IH3034 (D), IH3036 (E), 536 (F), A21 (G), A1396 (H), and IH3095 (I). The DNAs were hybridized with nick-translated α-^{32}P-labeled DNA of probe D (Fig. 1). As a control *Bam*HI cleaved DNA of plasmid pANN801 was used (lane K). For size markers, *Bgl*II-cleaved λ DNA was used (lane L).

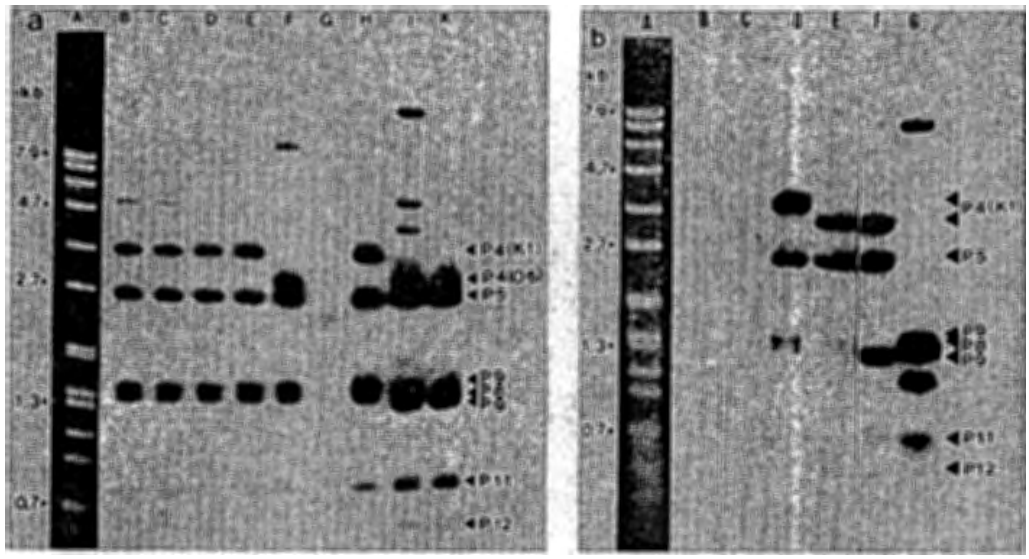

FIG. 4. Southern hybridization pattern of *Pst*I-cleaved total DNA as follows. (a) *E. coli* strains RS218 (B), RS226 (C), IH3034 (D), IH3036 (E), 536 (F), A21 (G), A1396 (H), and IH3095 (I) and plasmid pANN801 (K). (b) *E. coli* strains 5K (B), HB101 (C), A1521 (C), and A1523 (E) and plasmids pANN801-13 (F) and pPIL110-54 (G). The DNAs were hybridized with nick-translated, α-^{32}P-labeled DNA of probe A (Fig. 1). For size markers *Eco*RI-cleaved SPP1-DNA was used (lanes A). The different *Pst*I fragments were indicated as P4 through P12 (see the text).

Selection of *sfa*-specific DNA probes. The S fimbriae of the different strains were further characterized by DNA-DNA hybridization studies. For this reason DNA probes specific for the Sfa (*sfa*) determinant and some flanking regions were isolated. All six probes were derived from recombinant plasmids carrying *sfa*-specific sequences of the uropathogenic O6 *E. coli* strain 536 (9). As indicated by Tn*5* insertional mutants (10), probe A from Fig. 1 spans over a distance of 9 kb and represents the whole *sfa* determinant together with 2.5-kb DNA adjacent to the *sfa* coding region. Probes C and D carry *sfa* internal sequences only, and probes B, E, and F carry sequences which include the right (5′) and left (3′) boundaries of the *sfa* determinant.

Identification of one *sfa* determinant on the chromosome of *E. coli* wild-type strains. To determine the number of *sfa* copies on the chromosomes of Sfa$^+$ strains we cleaved chromosomal DNA of the strains with *Bam*HI and *Sal*I, which do not cleave the cloned *sfa* determinant of strain 536 (Fig. 1). The DNAs were hybridized against radioactively labeled probe D, which carries internal *sfa*-specific sequences. All O18:K1 (RS218, RS226, IH3034, IH3036) and O6:K$^+$ (536, IH3095) strains tested (Fig. 3, lanes B through F and I) gave positive hybridization signals in one particular band ranging from 14 to 18 kb. Similar results were obtained with the O83:K1 Sfa$^+$ strains (data not shown). The DNAs isolated from the Sfa-negative strains IH3036 (O18:K1) and A1396 (O83:K1; Fig. 3, lanes E and H) hybridized likewise when probed against the labeled *sfa*-specific fragment, which strongly suggests that the *sfa* determinant is still present on the chromosome of these Sfa$^-$ strains. DNA isolated from the Sfa$^-$, P$^+$ O7:K1 isolate A21 and from the two nonfimbriated K-12 strains HB101 and 5K gave no positive signals after hybridization with probes D (Fig. 3, lanes A and G) and A (Fig. 4b, lanes B and C).

Physical structure of the *sfa* coding regions in different *E. coli* wild-type strains. The *sfa* coding region of strain 536 (O6:K15:H31) is cleaved by the restriction endonuclease *Pst*I into six fragments, which have been termed P5, P9, P8, P11, P12 and P4 (10). From Fig. 1 it is obvious that these *Pst* I fragments are included in the DNA probe A, which represents an *Eco*RV fragment spanning the whole *sfa* determinant (see above). To find out whether the different wild-type strains have *sfa* determinants comparable to that of *E. coli* 536, their total DNAs were cleaved with *Pst*I and hybridized against the radioactivity labeled probe A. The six *Pst*I fragments P5 through P4 were visible in the DNAs prepared from both O6:K$^+$ strains (536, IH3095; Fig. 4a, lanes F and I). The differences among the O6 strains in the sizes of the P9 fragments seem to result from the appearance of an additional *Pst*I site in the P9 fragment of strain IH3095, compared with 536, which resulted in a smaller P9 fragment in IH3095. This *Pst*I site, which also is present in the cloned *sfa* sequence (Fig. 1), is masked in the 536 wild-type strain (J. Hacker, P. Stolt, and W. Goebel, manuscript in preparation). The longer-sized fragment (termed P9′) was also detected in the DNA of the O18:K1 (RS218, RS226, IH3034, IH3036) and O83:K1 (A1396, A1521, A1523) strains. In these DNAs (including the Sfa-negative variants IH3036 and

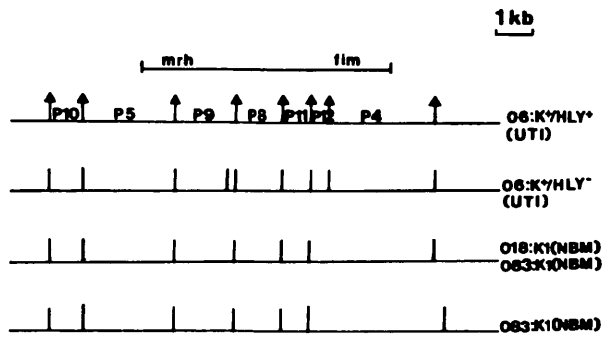

FIG. 5. Physical map of the *sfa* region of different pathogenic *E. coli* serotypes based on the presence of *Pst*I recognition sites.

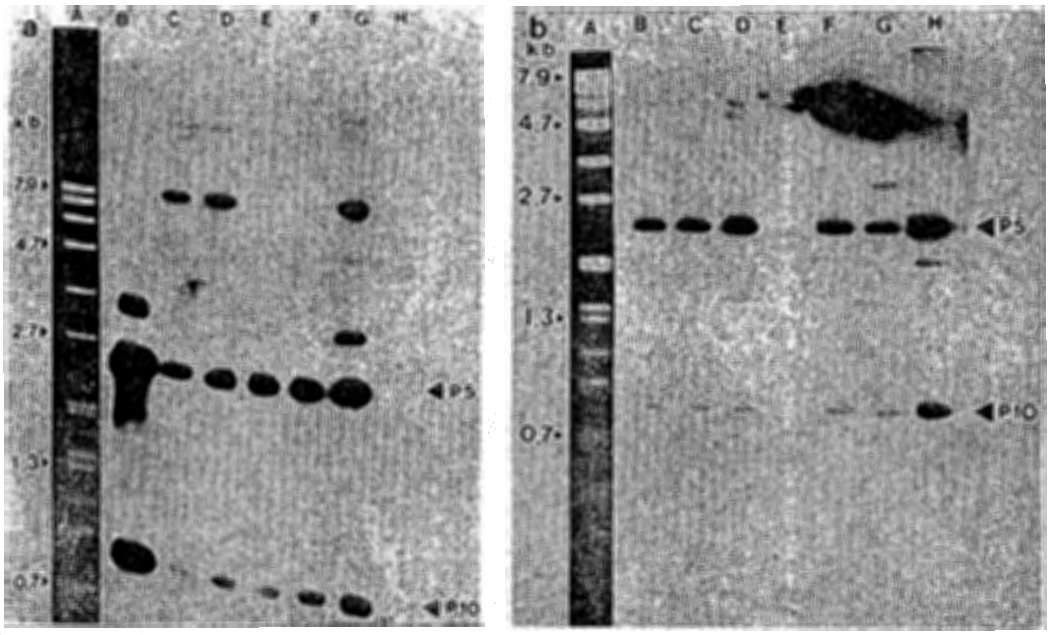

FIG. 6. Southern hybridization of *Pst*I-cleaved total DNA as follows. (a) *E. coli* strains RS218 (C), RS226 (D), IH3034 (E), IH3036 (F), 536 (G), and HB101 (H) and plasmid pANN801-13 (B). (b) *E. coli* strains A1521 (B), A 1523 (C), IH3095 (F), and 536 (G) and plasmid pANN801 (H). The DNAs were hybridized with nick-translated α-^{32}P-labeled DNA of probe B (Fig. 1). For size markers *Eco*RI-cleaved SPP1 DNA was used (lanes A).

A1396) the fragment P12 was missing, whereas the other *Pst*I fragments were still visible (Fig. 4a, lanes B through E and H, Fig. 4b, lanes D and E). Thus the *Pst*I sites at the coordinates 4.5 and 8.2 kb (Fig. 1) differed among the various strains. Because the P12 fragment is located adjacent to P4, the latter fragment differed in size between the O6:K1$^+$ and K1 strains (termed P4-O6 and P4-K1; Fig. 4a). In the O83:K1 strains the P4 fragment differed likewise (Fig. 4b).

The results obtained with the DNA probe A have been summarized in Fig. 5. They were further confirmed by the use of the probes C and D which carry internal sequences of the *sfa* coding region and probe E (Fig. 1). As expected the *Pst*I fragments P5, P9 (or P9'), and P8 appeared after hybridization of the chromosomal DNAs against probe C. P8, P11, and P12 (in the case of O6 strains) were visible after using probe D. The P12 fragment (in the case of O6 strains) and the different P4 fragments, which differed in size between the O6 and the K1 strains, were detected when the probe E was used (data not shown; Fig. 4).

Physical structure of the *sfa*-flanking regions in *E. coli* wild-type strains. After hybridization of the *Pst*I-cleaved chromosomal DNAs with probe A, an identical *Pst*I fragment, P5, was still visible in the hybridization pattern from all DNAs tested (Fig. 4). The *sfa* determinant is transcribed from a promoter located on the *Pst* I fragment P4. Transcription is terminated in fragment P5 (17; Hacker et al., *Molecular Biology of Microbial Pathogenicity*, in press; Schmoll and Jarchau, unpublished observations) (Fig. 1). P5 contains the 3' end of the *sfa* determinant. The *Pst*I site flanking P5 to the left is located 1.3 kb downstream of the *sfa* coding region. It thus follows from the hybridization pattern of Fig. 4 that not only the *sfa* determinant itself but also additional *sfa*-flanking sequences were conserved in the different strains. This finding was further confirmed by probe B representing more than 3 kb of left flanking sequences. For the O18:K1 strains RS218, RS226, IH3034, and IH3036 and the O6:K15 strain 536 (Fig. 6a, lanes B through G) the *Pst*I fragment P10 (adjacent to P5) was also completely conserved, and additional homology extended to the next *Pst*I fragment located at the left of P10. The homology between the different strains in the P4 fragment (Fig. 4), which includes more than 2 kb of the 5' upstream *sfa* region, was further confirmed by the use of probe F (data not shown).

No further differences were found between the Sfa$^+$ and the Sfa$^-$ strains in the *sfa* determinant and the regions which were located next to the coding sequences (Fig. 6a, lane F; Fig. 6b, lane D). With probes that represent flanking regions of the *sfa* determinant (probes A, B, F) some minor bands were visible after Southern hybridization, which seems to indicate further homology between these sequences and other chromosomal regions (Fig. 4 and 6).

The idea that not only the *sfa* determinant itself but also its flanking regions represent DNA sequences specific for a limited number of pathogenic *E. coli* serogroups was additionally supported by the fact that DNA isolated from strains HB101 (K-12) and A21 (O7:K1) did not hybridize with probes B and F (Fig. 6a, lane H, Fig. 6b, lane E).

Cross-homology between *sfa* and F1C determinants. F1C fimbriae cross-react specifically with antiserum raised against S fimbriae (Fig. 2). In addition, homology between the two determinants was observed on the DNA level. *Pst*I-cleaved DNA of the F1C specific recombinant plasmid pPIL110-54 reacted in distinct bands when probed against the radioactively labeled *sfa* probe A (Fig. 1). *Pst*I fragments with similar sizes, such as fragments P12, P11, and P8 of the *sfa* determinant, were visible in the autoradiogram (Fig. 4b, lane G; see Discussion). The same fragments were labeled

when the internal *sfa* probe D was used (data not shown), indicating that at least parts of both determinants were similar in their genetic structure.

DISCUSSION

Adhesion factors which cause attachment of *E. coli* bacteria to epithelial cells can be isolated from different types of strains: MS fimbriae, which use a mannose-containing receptor, are well distributed among all *E. coli* serotypes. P fimbriae, which recognize a Gal-Gal globoside, can be isolated from a limited number of uropathogenic *E. coli* clones (30, 38). The third group, sialic acid-recognizing S-fimbrial adhesins were predominantly detected on uropathogenic O6:K$^+$ strains and with a high incidence on meningitis isolates (21). As shown here not only O18:K1 strains but also O83:K1 meningitis isolates exhibit the Sfa.

MS fimbriae mostly belong to the distinct serological group F1A (28), and they are homologous to each other (4, 5). In contrast the P fimbriae differ in terms of their serological specificity, and at least eight different serogroups (F7 through F13) have been described (30). S fimbriae on O18:K1 strains are serologically homologous (19), and our results extend this observation to S fimbriae of O83:K1 and O6:K$^+$ strains (Fig. 2). As shown previously (19), S fimbriae differ serologically from P and MS fimbriae, but cross-react with F1C fimbriae as was found in the present study. Such relations were also observed between different P and MS fimbriae (6). In this respect, it is interesting to note that the two Sfa that have been characterized, from IH3084 (an R:K1:H47 strain) (22) and from the strain 536 (O6:K15:H31) used here, are not serologically identical but share high cross-reactivity (A. Pere, unpublished results).

Homology between S-fimbrial determinants from uropathogenic and meningitis strains was also observed at the DNA level. With specific DNA probes which represented internal regions of the *sfa* determinant a high degree of conservation was found in the genome structures. As summarized in Fig. 5 on the basis of *Pst*I recognition sites, only marginal alterations between O6:K$^+$ and O18:K1 as well as O83:K1 strains were detected. The differences between the hemolysin-positive (Hly$^+$) and hemolysin-negative (Hly$^-$) O6 strains found in the P9 region (map position 4.5) (Fig. 1), however, seem to result from an additional *Pst*I site observed in the Hly$^-$ strain but not in the Hly$^+$ strain. The disappearance of the *Pst*I site results from a masking of this sequence in the O6 Hly$^+$ wild-type strain, presumably via a specific DNA modification process which is also absent in the *E. coli* K-12 strains (Hacker et al., manuscript in preparation). We do not know at this moment whether this modification event has an influence on the regulation of gene expression. The region which is specifically modified does not code for the fimbriae itself but seems to be involved in the adhesin production of the strain.

Furthermore differences between the various strains exist in the P12/P4 region (Fig. 5). The P12 fragment carries parts of the *sfaA* gene, which codes for the fimbrial structural protein subunit (10; Schmoll, Hacker, and Goebel, submitted for publication), and P4 includes the 5'-flanking region of the *sfa* determinant. The differences between the strains result from a change in the location of the *Pst*I site flanking P4 to the right (Fig. 4b) in one O83:K1 strain and from the absence of two *Pst*I sites (lying 50 base pairs adjacent to each other) at map position 8.2 (Fig. 1) in the K1 strains. The presence of the two *Pst*I sites in the O6:K$^+$ strains and their absence in the O18:K1 and O83:K1 isolates indicate sequential differences in the structural fimbrial genes which may be also the cause of the incomplete cross-reactivity in the S-fimbrial proteins in quantitative enzyme-linked immunosorbent assays (A. Pere, unpublished results). One of the two *Pst*I sites mentioned above is absent also in the F1C determinant (39), resulting in a 50-base-pair increased P12 fragment of the F1C determinant after hybridization with a *sfa* specific probe (Fig. 4b). Nevertheless more than 70% homology exists on the basis of the DNA sequences of the fimbrial subunit-coding genes of the two determinants (39; Schmoll et al., submitted for publication), which corresponds well with the data presented in this study (Fig. 2 and 4b).

The genetic homology between the various *sfa* determinants and between *sfa* and F1C is contradictory to the situation found for the P determinants, which vary to a great extent (24, 36). Additionally the number of determinants located on the chromosomes differs between P- and S-fimbriated strains. Whereas uropathogenic strains very often harbor more than one P determinant (13, 14; G. Boulnois and K. Jann, personal communication), only one *sfa* determinant was detected on the chromosomes of each S-fimbriated isolate. These findings were expected because the possession of several copies of the identical *sfa* determinant would not be profitable for the host strains. In such a case their turn on and off would not modulate their antigenic characteristics, a fact which seems to be true in the case of P fimbriae differing in their antigenicity.

Neither the *sfa* coding region nor the DNA sequences adjacent to the determinant reveal any homology to *E. coli* K-12 DNA and to DNA isolated from an Sfa$^-$ *E. coli* wild-type strain. Thus it seems that the *sfa* region is not usually found in the *E. coli* genome but seems to represent a DNA cluster specific for particular pathogenic serotypes like O18:K1, O83:K1, or O6:K$^+$. It is also true that only a few but not all of the meningitis clonal groups possess the *sfa* determinant, indicated by the lack of the *sfa* genes in an O7:K1 meningitis isolate. Indeed, recent analysis of the population structure of *E. coli* strains associated with meningitis and septicemia (33) showed that most of the Sfa$^+$ strains form a genetically related clonal group. Similar observations have been made for a region bearing the hemolysin (*hly*) determinant which also is present in particular uropathogenic *E. coli* strains but absent in K-12 and other nonhemolytic wild-type isolates (17).

Variants of Sfa$^+$ strains have been isolated which exhibited an Sfa$^-$ phenotype (9, 27). As has also been demonstrated for the uropathogenic O6:K15 strain 536 (17) the *sfa* genes were still present on the chromosome of Sfa$^-$ O18:K1 and O83:K1 isolates, and the same has been found for MS fimbriae. For MS fimbriae the molecular basis of phase variation has been worked out. An invertible element in front of the determinant and a gene coding for a negatively regulating protein seem to be involved in the regulation of MS pilus expression (1, 28).

For another virulence determinant coding for the *E. coli* hemolysin the molecular events which lead to negative phenotypes were well established for the uropathogenic O6:K15 strain 536 (9, 17, 18). Large deletions including the *hly* structural genes lead to Hly$^-$ variants, which had also lost the ability to produce S fimbriae. The use of O18:K1 hemolytic and nonhemolytic strains differing with respect to their expression of S fimbriae may allow us to determine the extent to which such a linkage between different virulence factors is of general importance in the regulation of *E. coli* virulence.

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