

## Gene Clusters for S Fimbrial Adhesin (*sfa*) and F1C Fimbriae (*foc*) of *Escherichia coli*: Comparative Aspects of Structure and Function

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Received 10 December 1987/Accepted 11 June 1988

Fimbrial adhesins enable bacteria to attach to eucaryotic cells. The genetic determinants for S fimbrial adhesins (*sfa*) and for F1C ("pseudotype I") fimbriae (*foc*) were compared. *Sfa* and F1C represent functionally distinct adhesins in their receptor specificities. Nevertheless, a high degree of homology between both determinants was found on the basis of DNA-DNA hybridizations. Characteristic differences in the restriction maps of the corresponding gene clusters, however, were visible in regions coding for the fimbrial subunits and for the S-specific adhesin. While a plasmid carrying the genetic determinant for F1C fimbriae was able to complement transposon-induced *sfa* mutants, a plasmid carrying the genetic determinant for a third adhesin type, termed P fimbriae, was unable to do so. Proximal *sfa*-specific sequences carrying the S fimbrial structural gene were fused to sequences representing the distal part of the *foc* gene cluster to form a hybrid cluster, and the *foc* proximal region coding for the structural protein was ligated to *sfa* distal sequences to form a second hybrid. Both hybrid clones produced intact fimbriae. Anti-F1C monoclonal antibodies (MAbs) only recognized clones which produced F1C fimbriae, and an anti-S adhesin MAb marked clones which expressed the S adhesin. However, one of four other anti-S fimbriae-specific MAbs reacted with both fimbrial structures, S and F1C, indicating a common epitope on both antigens. The results presented here support the view that *sfa* and *foc* determinants code for fimbriae that are similar in several aspects, while the P fimbriae are members of a more distantly related group.

Fimbriae or pili are bacterial cell wall appendages which consist of about 1,000 identical protein subunits. A single fimbria is 7 nm in diameter and up to 1  $\mu$ m in length (9, 17). Very often fimbriae are associated with adhesins which enable the bacteria to attach to eucaryotic cells, including erythrocytes (RBC) (28, 31). Adherence of bacteria to eucaryotic cells is a prerequisite for many infectious diseases (27, 30, 41).

Extraintestinal *Escherichia coli* isolates cause infections of the urinary tract and the blood (sepsis) and are also the causative agents of newborn meningitis (32). Such *E. coli* isolates may carry different types of fimbrial adhesins. P fimbriae recognize an  $\alpha$ -D-galactosyl-(1-4)- $\beta$ -D-galactose receptor. They are strongly associated with uropathogenic *E. coli* strains and can be further subdivided into nine serologically distinct groups (F7<sub>1</sub> through F13) (16, 31, 41). The P fimbrial gene clusters of different serogroups are functionally related. This was shown by the construction of hybrid clones consisting of DNA sequences derived from different P determinants (*cis*-complementation) and also by the fact that P-specific gene products were able to produce a wild-type phenotype of P insertion mutants (*trans*-complementation) (24, 36, 43, 45). Another group of *E. coli* gene clusters, coding for type I fimbrial adhesins which interact with  $\alpha$ -D-mannose-containing receptors, are also completable by each other (5-7).

S fimbrial adhesins (Sfa) interact with  $\alpha$ -sialyl-(2-3)- $\beta$ -D-galactose-containing receptor molecules (21, 35). They are associated with urinary tract infection strains and, to a larger extent, newborn meningitis with *E. coli* isolates (22). The genetic determinant coding for Sfa has been cloned from the chromosome of a uropathogenic *E. coli* O6 strain (13).

Recently, the S adhesin molecule, a protein of 12 kilodaltons (kDa), was isolated and characterized, and the sequence of the *sfaA* gene, coding for the S fimbrial protein subunit, was determined (28, 39). The *sfa* determinants from different strains have high sequence homology (33). Surprisingly, it was found that the *sfa* determinant is also related to another gene cluster (*foc*) coding for F1C fimbriae (34). In contrast to Sfa, however, F1C fimbriae are not able to agglutinate RBC (18, 44).

In this contribution, we present evidence that the *sfa* and *foc* determinants belong to the same general group of fimbrial gene clusters. The structural homology of the determinants and the serological relatedness of the corresponding proteins were demonstrated by the use of specific gene probes and monoclonal antibodies (MAbs). It is further shown that *foc*-specific gene products are able to produce a wild-type phenotype in *sfa* insertion mutants and that hybrid DNAs consisting of *sfa*- and *foc*-specific sequences code for intact fimbriae after transformation into nonfimbriated *E. coli* K-12 strains.

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

**Bacterial strains and recombinant DNAs.** The wild-type strain 536 (O6:K15) exhibits the S fimbrial adhesin and type

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TABLE 1. Recombinant DNAs

Recombinant DNA	Genotype	Characteristics	Reference
pANN801-13	<i>sfa</i> <sup>+</sup> Ap <sup>r</sup>	<i>sfa</i> determinant (4.1–13.9 kb) <sup>a</sup>	13
pANN801-11	$\Delta$ <i>sfa</i> Ap <sup>r</sup>	Distal part of the <i>sfa</i> determinant (4.1–9.3 kb)	13
pANN801-15	$\Delta$ <i>sfa</i> Cm <sup>r</sup>	Proximal part of the <i>sfa</i> determinant (11.1–13.7 kb)	33
pANN801-13-1	$\Delta$ <i>sfa</i> Ap <sup>r</sup>	Proximal part of the <i>sfa</i> determinant (9.3–11.1 kb)	33
pMP13-Sph	$\Delta$ <i>sfa</i>	Proximal part of the <i>sfa</i> determinant (11.2–11.8 kb)	34
pANN1E6	<i>sfa</i> Ap <sup>r</sup>	<i>sfa</i> determinant, <i>sfa</i> -flanking region, cosmid	Present study
pPIL110-54	<i>foc</i> <sup>+</sup> Cm <sup>r</sup>	<i>foc</i> determinant (0.0–13.0 kb)	44
pPIL110-512	<i>foc</i> <sup>+</sup> Ap <sup>r</sup>	<i>foc</i> determinant (5.9–7.8 kb)	44
pANN921	F8 Ap <sup>r</sup>	F8 determinant	12
pRHU845	F13 ( <i>pap</i> <sup>+</sup> ) Tc <sup>r</sup>	F13 determinant	29
pANN801-C25	<i>foc sfa</i> Cm <sup>r</sup>	Hybrid	Present study
pANN801-E4	<i>sfa foc</i> Ap <sup>r</sup>	Hybrid	Present study

<sup>a</sup> The coordinates are given for *sfa* and *foc* determinants in Fig. 1.

I fimbriae and has been described elsewhere (11). The O18:K5 strain 2980 produces P fimbriae (serogroup F8) and F1C fimbriae; strain A21 (O7:K1) exhibits P fimbriae. The strains were described previously (15, 33, 46). For transformation and as controls in Southern hybridizations, the *E. coli* K-12 strains HB101 and 5K were used. The recombinant DNAs used in this study are listed in Table 1.

**Recombinant DNA techniques.** Chromosomal and plasmid DNA were isolated as described earlier (4, 10, 19). For restriction analysis, DNA was treated with appropriate restriction enzymes, and the resulting fragments were separated by gel electrophoresis on 0.7 to 1.0% agarose gels as described before (19). DNA fragments were isolated after agarose gel electrophoresis by electroelution (26). For cloning, DNA fragments were ligated into suitable vector molecules after heat inactivation of the restriction endonucleases at 65°C for 6 min as described before (3). Recombinant cosmid DNAs were selected from a recombinant cosmid gene bank of strain 536 by colony dot blot as described

before (26). *E. coli* K-12 strains were transformed by the CaCl<sub>2</sub> method (23).

**Generation of DNA probes.** The *sfa*-specific gene probes are shown in Fig. 1. Probes A (coordinates, 0 to 4.4 kilobases [kb], Fig. 1), F (11.4 to 14.4 kb), and G (13.9 to 20.3 kb) were generated from the cosmid DNA pANN1E6. Probes B (4.1 to 7.1 kb) and C (7.1 to 9.3 kb) were derived from plasmid pANN801-11, probe D (9.3 to 11.1 kb) was from plasmid pANN801-13-1, and probe E (11.2 to 11.8 kb) was from plasmid pMP13-Sph (see Table 1). Only probes which were eluted from agarose gels were used in Southern hybridizations.

**Nick translation, Southern hybridization, and autoradiography.** The DNA probes were labeled by nick translation with a mixture of all four  $\alpha$ -<sup>32</sup>P-labeled deoxynucleoside triphosphates as described before (37). Transfer of DNA fragments from agarose gels to nitrocellulose filters and washing and autoradiography of the filters after hybridization were performed as described previously (40). The filters

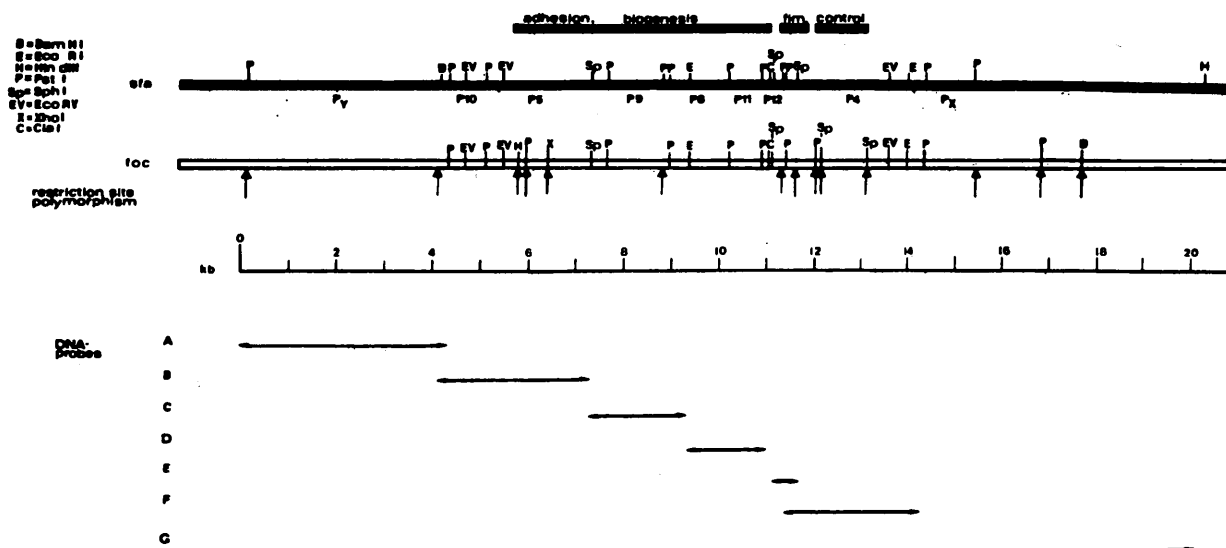


FIG. 1. Detailed restriction maps of *sfa* and *foc* determinants and their flanking regions. The *Pst*I fragments of the *sfa* determinants are indicated by numbers below the heavy line representing the *sfa* determinant. *EcoRV*, *Sph*I, *Cla*I, *Eco*RI, and *Xho*I sites are only shown for the coding regions. Differences between *sfa* and *foc* in restriction sites are indicated by arrows below the open bar representing the *foc* determinant. The DNA probes used in Southern hybridizations are indicated by the lines at the bottom (probes A to G). The functional regions of the *sfa* determinant are indicated at the top.

were hybridized in 50% formamide at 43°C for 3 days. Stringent conditions were used for the washing procedure: 30 min at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) and then four times for 30 min each at 56°C in 0.1× SSC–0.1% SDS.

**Preparation of fimbriae.** Fimbriae were isolated from bacteria grown on plates as described previously (15).

**SDS-polyacrylamide gel electrophoresis of fimbrial protein subunits.** After fimbriae were disintegrated by boiling for 5 min in 10 mM Tris hydrochloride (pH 7.8) containing 4% SDS, 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 protein) were run on slab gels as described previously (15, 46).

**Preparation of polyclonal and monoclonal antisera.** Polyclonal fimbria-specific antisera were prepared from rabbits with purified fimbrial protein eluted from an SDS-polyacrylamide electrophoresis gel (15). The preparation and characterization of the monoclonal antisera used were described elsewhere (1, 8, 28, 38).

**Determination of fimbria antigens.** Fimbriated cells were characterized by agglutination with antisera on glass slides. Western blots (immunoblots) were carried out by the method of Towbin et al. (42).

**Immunoelectron microscopy.** For immunoelectron microscopy, bacteria were transferred to copper grids coated with polyvinyl-Formvar. Incubation with MAbs and goat anti-mouse immunoglobulin G-coated gold spheres was described previously (28).

**Determination of adhesins.** S-specific adhesion was determined after mixing the bacterial cells with human and bovine RBC with and without 2% mannose and with RBC treated with neuraminidase (13, 21).

## RESULTS

**Genetic structure of *sfa* and *foc* determinants.** The genetic determinants coding for S fimbrial adhesins (*sfa*) and F1C fimbriae (*foc*) were cloned on the recombinant plasmids pANN801-13 and pPIL110-54, respectively (13, 44). Recently it was shown by DNA-DNA dot blot hybridization studies that both gene clusters are structurally related (34). In order to demonstrate this relatedness more precisely, we generated five DNA probes (probes B to F, Fig. 1) which are specific for the *sfa* coding region. These probes were used in Southern hybridizations against *sfa*-, *foc*-, and P-specific recombinant DNAs (Fig. 2).

The *sfa* determinant was cleaved by the restriction enzyme *Pst*I into six fragments, P5, P9, P8, P11, P12, and P4. The main direction of transcription of the *sfa* determinant was from P4 to P5 (Fig. 1) (13). The *Pst*I-cleaved plasmid DNAs pANN801-13, pANN921 and pPIL110-54, coding for S fimbrial adhesins, P fimbriae, and F1C fimbriae, respectively, were hybridized against probe B, which represents the distal (3') end of the *sfa* determinant. This region, in the case of *sfa*, codes for the S-specific adhesin (Fig. 1) (13, 28). It was demonstrated (Fig. 2) that the *Pst*I fragment P5 of *sfa* (lane A) disappeared in *foc* DNA, and two *foc*-specific fragments ( $\Delta$ P5) were visible (lane B), indicating the presence of an additional *Pst*I site in the *foc* determinant compared with *sfa*. As summarized in Fig. 1, similar differences between *sfa* and *foc* determinants were found in that region on the basis of the sites for the restriction enzymes *Hind*III and *Xho*I.

After hybridization of *sfa*- and *foc*-specific DNAs with probes C, D, E, and F (Fig. 2 and data not shown), positive

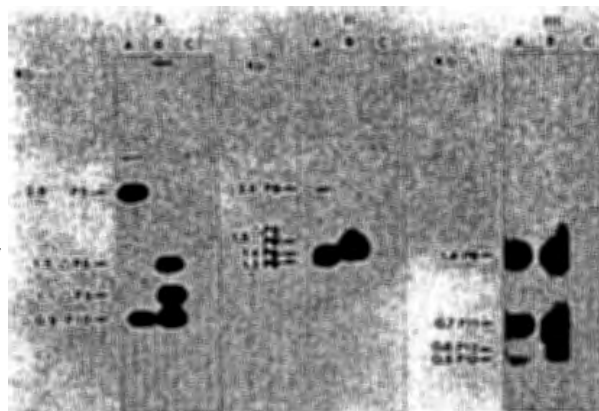


FIG. 2. Southern hybridization pattern of *Pst*I-cleaved DNA of plasmids pANN801-13 (*sfa*<sup>+</sup>; lanes IA, IIA, and IIIA); pPIL110-54 (*foc*<sup>+</sup>, lanes IB, IIB, and IIIB), and pANN921 (P-specific; F8, lanes IC, IIC, and IIIC; see Table 1). The DNAs were hybridized with nick-translated  $\alpha$ -<sup>32</sup>P-labeled DNA of probes B (blot I), C (blot II), and D (blot III) (see Fig. 1).

signals occurred in all cases tested. Only a few differences in restriction sites between *sfa*- and *foc*-specific DNAs (e.g., the absence of one *Pst*I site in *foc* compared with *sfa*) were visible in the regions corresponding to probes C and D, which code for proteins of 20, 26, and 90 kDa, involved in the biogenesis of the fimbriae (13; Ludwig and Hacker, unpublished results). The regions coding for fimbrial subunits (probe E and part of probe F) of both determinants, however, consisted of more heterogeneous stretches of DNA sequences, indicated in Fig. 1 (39, 44).

In contrast to the *foc* sequences, which hybridized to all *sfa*-specific probes used, the P-specific DNA showed no significant homologies with *sfa* probes B to E. However, homology was seen between P-specific sequences and *sfa* probe F (specific for the region involved in the control of transcription). A similar observation was also made recently by Ott et al. (34). These data correspond to the fact that about 1 kb of DNA of the control region of *sfa* and the P-specific determinant *pap* showed sequence homology of up to 90% in recent DNA sequence studies (2; T. Schmoll, W. Goebel, and J. Hacker, submitted for publication).

**Genetic structure of *sfa* and *foc* flanking regions.** In order to compare the DNA regions located adjacent to the *sfa* and *foc* gene clusters, an *sfa*-specific recombinant cosmid, pANN1E6, was isolated from a cosmid gene bank of strain 536 (20). pANN1E6 consists of the vector pHC79, the *sfa* determinant 6.5 kb in size, and DNA sequences flanking the *sfa* coding region at its proximal and distal ends. From these flanking DNA sequences, the DNA probes A (4.4 kb in size and specific for the 3'-flanking region) and G (9 kb in size and specific for the 5'-flanking region) were generated. These probes were hybridized against chromosomal DNAs isolated from strains producing F1C, P, or type I fimbriae.

The data presented in Fig. 3 clearly show that *Pst*I- (Fig. 3-I, lanes B and E) and *Bam*HI- (Fig. 3-II, lanes A and E) cleaved DNAs isolated from the F1C-positive strain 2980 and from the Sfa-producing wild-type strain 536 produced specific bands after hybridization against radioactively labeled probes A and G. In contrast, DNAs isolated from a P fimbria-specific strain (A21) and from two *E. coli* K-12 laboratory strains, which, in the case of strain 5K, exhibit type I fimbriae, gave no significant signals after being probed

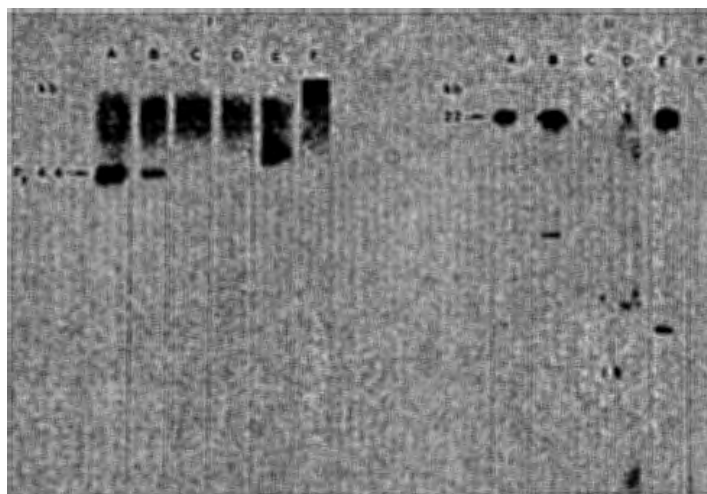


FIG. 3. Southern hybridization pattern of *Pst*I-cleaved (blot I) and *Bam*HI-cleaved (blot II) total DNA of strains HB101 (pcos1E6) (*sfa*<sup>+</sup>; lanes IA and IIA), 536 (*sfa*<sup>+</sup>; lanes IB and IIB), 5K (type I specific; lanes IC and IIC), A21 (P-specific; lanes ID and IID), 2980 (*foc*<sup>+</sup>; P-specific; lanes IE and IIE), and HB101 (lanes IF and IIF). The DNAs were hybridized with nick-translated  $\alpha$ -<sup>32</sup>P-labeled DNA of probes A (blot I) and G (blot II); see Fig. 1.

with the DNAs representing *sfa*-specific flanking sequences. Therefore it can be concluded that not only the *sfa* and *foc* coding regions but also their flanking sequences, at least 12 kb (proximal) and 7 kb (distal to the coding regions) in size, showed strong homologies. These flanking sequences are not present on the chromosomes of strains carrying gene clusters coding for P and type I fimbriae.

**trans-Complementation studies.** In order to find out whether the structural homology between *sfa* and *foc* gene clusters has consequences for their functional behavior, *trans*-complementation studies were carried out. In these experiments, P- and F1C-specific DNAs were tested for their ability to produce an Sfa<sup>+</sup> wild-type phenotype from Sfa<sup>-</sup> insertion mutants. Therefore, *E. coli* K-12 strains harboring transposon-induced mutations of the *sfa*-specific plasmid pANN801-13 were cotransformed with the *foc*-specific DNA pPIL110-54 and with the P-specific DNA pRHU845. The mutant DNAs carry Tn1000 insertions in the *sfa* coding region of the *Pst*I fragments P9, P8, and P11 (Fig. 1). These sequences are responsible for the biogenesis of fimbrial adhesins (13, 34; Hacker and Ludwig, unpublished results). The different DNAs were able to coreplicate in the same cell because the pANN801-13 derivatives carry pBR322 sequences, whereas pPIL110-54 and pRHU845 consist of the vector molecule pACYC184 together with fimbria-specific insert DNAs (29, 44).

Table 2 summarizes the results of the experiments. The *sfa*-specific insertion mutants (pANN801-13/Tn1000-56, -57, -58) were complemented by the *foc*-specific plasmid pPIL110-54. This was shown by the expression of the S-specific fimbriae and an S-specific adhesion by the double transformants. The ability to express functions of the *sfa* determinant was restricted to the *foc*-specific sequences. Complementation was absent with cotransformants carrying the P-specific DNA pRHU845 and the *sfa* insertion mutants. Therefore it can be concluded that the *sfa* and *foc* but not P coding determinants are related at the functional level.

**Construction of hybrid plasmids carrying *sfa* and *foc* coding sequences.** In order to study further the functional relatedness of the *sfa* and *foc* determinants, hybrid plasmids containing DNA fragments derived from both gene clusters

were constructed. The *sfa*-specific DNA pANN801-11, which carries a *Bam*HI-*Eco*RI fragment (coordinates 4.0 to 9.3 kb, Fig. 1) that includes the *Pst*I fragments  $\Delta$ P8, P9, and P5, was cleaved with the enzyme *Eco*RI. pANN801-11 represents the distal (3' end) region of the *sfa* determinant coding for the adhesive properties of the corresponding antigen. The *foc*-specific *Eco*RI fragment of pPIL110-512 (coordinates 9.3 to 13.9 kb) carrying a *focA* structural gene was ligated into pANN801-11, resulting in a hybrid plasmid, pANN801-E4. pANN801-E4 consists of the vector pBR322, the adherence-specific region of the *sfa* determinant including the gene *sfaS*, and the *foc*-specific sequences coding for the F1C fimbriin protein FocA (Fig. 4).

In addition, the *sfa*-specific subcloned DNA pANN801-15, carrying the fimbriin-coding gene *sfaA* of the *sfa* gene cluster on a *Cla*I-*Eco*RV fragment (coordinates 11.1 to 13.7 kb) inserted into pACYC184, was cleaved with the enzyme *Cla*I. A *foc*-specific *Cla*I fragment (coordinates ca. 0.0 to

TABLE 2. *trans*-Complementation between Tn1000-induced mutants of pANN801-13 (*sfa*<sup>+</sup>), pPIL110-54 (*foc*<sup>+</sup>), and pRHU845 (*pap*<sup>+</sup>)

Recombinant DNA(s)	S fimbriae production <sup>a</sup>	S-specific adhesion <sup>b</sup>
pANN801-13	+	+
pANN801-13/Tn1000-56	-	-
pANN801-13/Tn1000-57	-	-
pANN801-13/Tn1000-58	-	-
pPIL110-54	-	-
pRHU845	-	-
pANN801-13/Tn1000-56, pPIL110-54	+	+
pANN801-13/Tn1000-57, pPIL110-54	+	+
pANN801-13/Tn1000-58, pPIL110-54	+	+
pANN801-13/Tn1000-56, pRHU845	-	-
pANN801-13/Tn1000-57, pRHU845	-	-
pANN801-13/Tn1000-58, pRHU845	-	-

<sup>a</sup> S fimbriae were detected by Western blots of fimbrial preparations isolated from *E. coli* clones blotted against monospecific anti-S polyclonal serum and by electron microscopy.

<sup>b</sup> Neuraminidase-susceptible hemagglutination of bovine RBC.

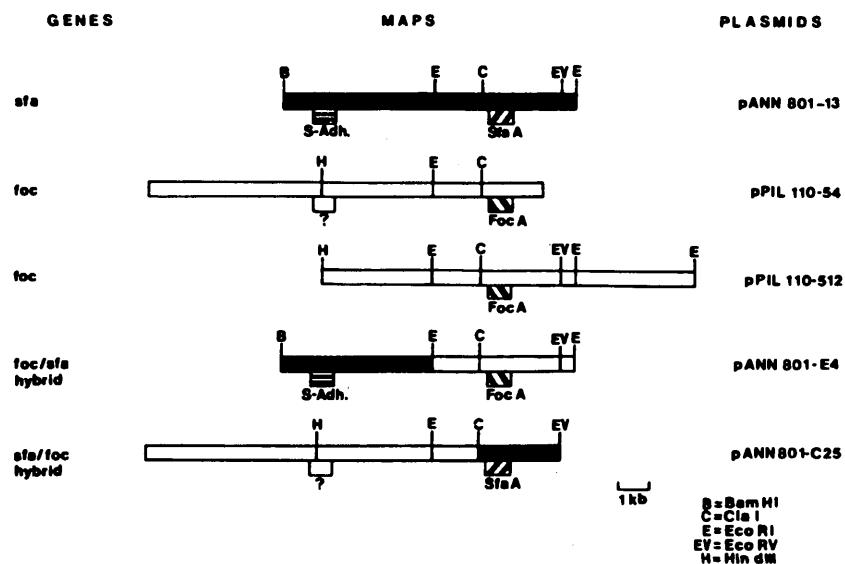


FIG. 4. Physical maps of the recombinant hybrid plasmids carrying *sfa*- and *foc*-specific DNA. The *sfa*-specific sequences are indicated by black bars, and the *foc*-specific DNA is marked by open bars. Fimbriae and adhesin genes are indicated by boxes below the bars. Only the cloning sites are shown. S-Adh, S adhesin.

11.1 kb) of plasmid pPIL110-54, representing the distal (3') part of the *foc* determinant, was ligated into the *Cla*I site of pANN801-15. The resulting hybrid plasmid, pANN801-C25, carries the fimbriillin-specific gene of *sfa* and the 3' end of *foc*. It was demonstrated by *Pst*I cleavage (data not shown) that the hybrid molecules carried the inserted DNAs in proper orientation (Fig. 4).

**Characterization of the fimbrial clones.** To answer the question of whether the recombinant clones produce intact fimbriae, the strains were analyzed by electron microscopy. As demonstrated in Fig. 5, the bacterial cells containing the parental *sfa*- and *foc*-specific DNA molecules or the hybrid plasmids were fimbriated. In order to identify the type of fimbriae produced, the cells were incubated with the F1C-specific MAb M9-6 (8) and then labeled with gold particles. As expected, the clones with a F1C-specific fimbrial subunit gene *focA* (pPIL110-54 and pANN801-E4) showed gold particles associated with the fimbriae, whereas the clones containing the S fimbrial structural gene *sfaA* did not. Identical results were obtained after using another F1C-specific MAb, F1-20025 (38) in Western blot experiments. Fimbriae prepared from the clones HB101(pPIL110-54) and HB101(pANN801-E4) reacted with the MAb in a protein band of 17 kDa (Fig. 6, blot II), which corresponds to the F1C protein subunit (44). In another Western blot (Fig. 6, blot I) analysis, the protein preparation of clones HB101(pANN801-13) and HB101(pANN801-E4) reacted with the S adhesin-specific MAb A1 in a band of 12 kDa. Therefore, one can conclude that the S-specific adhesin SfaS, which was described as a protein of 12 kDa very recently (28), is also produced by hybrid clones carrying the *focA* fimbriillin subunit gene.

In addition, four different antibodies (F1 to F4) which are specific for the native S fimbrial structure (28) were used to characterize the recombinant clones in agglutination tests. As shown in Table 3, three of these antibodies (F2 to F4) recognized clones HB101(pANN801-13) and HB101(pANN801-C25). The recombinant DNAs of these clones carry the gene *sfaA*, coding for the S fimbrial subunit, a

protein of 16 kDa (39). One MAb (F1), however, reacted with the S- and with the F1C-fimbriated clones, carrying the parental (pANN801-13, pPIL110-54) or the hybrid (pANN801-E4, pANN801-C25) DNA. Thus, MAb F1 seems to recognize an epitope common for S and F1C fimbriae.

## DISCUSSION

The two genetic determinants *sfa* and *foc*, coding for the S fimbrial adhesin and for F1C fimbriae, respectively, show similarities in their DNA sequence composition and exhibit common epitopes on their corresponding fimbrial proteins. In addition, *foc*- and *sfa*-specific DNA sequences were able to correct mutational defects of the corresponding gene cluster in *trans*- and *cis*-complementation experiments. Therefore it can be concluded that *sfa* and *foc* determinants form one main group of fimbrial determinants. In previous studies, two other general groups of fimbrial determinants were established on the basis of similar experiments: the gene clusters coding for P fimbriae and the determinants expressing type I fimbriae (5-7, 24, 36, 43, 45).

*Sfa* and F1C antigens, however, differ in their receptor specificities. While S fimbrial adhesins bind to the  $\alpha$ -sialic acid-(2-3)- $\beta$ -D-galactose residues of eucaryotic cell walls, F1C fimbriae do not bind to such substances (18, 21, 35, 44). On the other hand, it has been shown recently that F1C fimbriae are able to adhere to uroepithelial cells of human origin (Korhonen, personal communication). Therefore it is speculated that F1C fimbriae may recognize another receptor substance that is presumably slightly different from the S receptor molecule.

Very recently, a fimbrial determinant was cloned from the chromosome of an O75:K1 *E. coli* strain M. Pawelzik, J. Heesemann, J. Hacker, and W. Opferkuch, submitted for publication). This gene cluster, termed the "F1C-related fimbriae" (*sfr*) determinant, is also highly related to *sfa* and *foc* on the basis of DNA-DNA hybridizations and Western blots but is lacking the S-specific receptor-binding specificity. In addition, Lund et al. have described a fimbrial

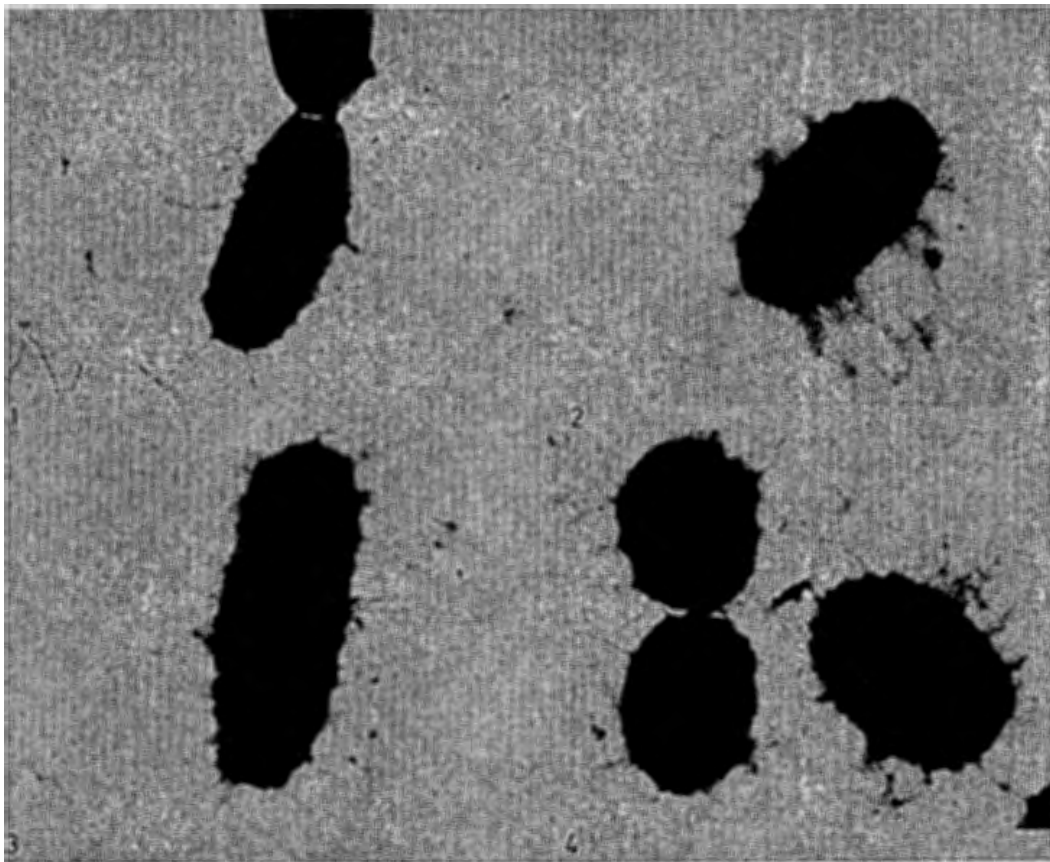


FIG. 5. Electron micrographs of *E. coli* K-12 strains carrying recombinant DNAs. The strains were incubated with F1C-specific MAb M9-6, followed by immunogold labeling and subsequent contrasting with 2% uranyl acetate. 1, HB101(pPIL110-54) (*foc*<sup>+</sup>); 2, HB101(pANN801-13) (*sfa*<sup>+</sup>); 3, HB101(pANN801-C25) (*sfa foc*); 4, HB101(pANN801-E4) (*foc sfa*).

determinant which is very similar to the *pap* gene cluster (P fimbriae of serogroup F13) in several aspects but codes for a different binding specificity (25). These data indicate that a shift in the receptor-binding specificity among one main

group of fimbrial adhesins is a general phenomenon not restricted to Sfa and F1C fimbriae.

Differences between S fimbrial adhesins and F1C fimbriae were also established for the fimbrial subunit protein itself. The DNA sequences of the corresponding structural genes (*sfaA* and *focA*) show 73.3% homology (39, 44), but they differ in regions coding for the hydrophilic parts of the proteins, which should be responsible for their serological specificities (14). Therefore it was not surprising that five of six F1C or S fimbriae-specific MABs only reacted with the homologous fimbriae (Table 3). One of these antibodies (MAB F1), however, recognized an epitope located on both fimbrial structures. This common epitope may be the reason for the serological cross-reactions between S and F1C fimbriae that were observed recently (33).

The antifimbriae MABs reacted with the corresponding fimbrial subunit proteins isolated from *E. coli* K-12 strains carrying original recombinant plasmids or hybrid DNAs (Table 3, Fig. 5 and 6). These hybrid plasmids consist of DNA sequences specific for the *sfa* and *foc* gene clusters. It is interesting that an MAB which is specific for the S adhesin (MAB A1 [28]) also reacted with a hybrid clone that included a plasmid of *foc*-specific DNA which codes for the F1C fimbrial protein and a DNA region which harbors the S-specific adhesin gene (*sfaS*). These data show that the S

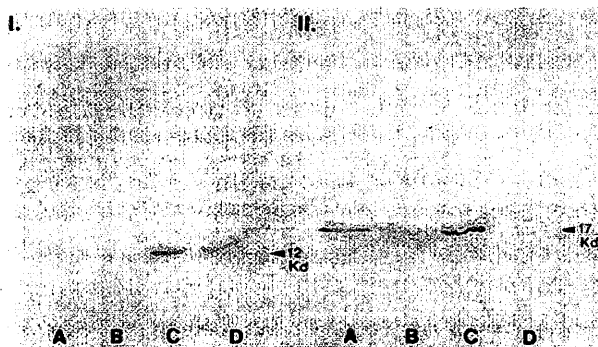


FIG. 6. Western blot of fimbrial protein preparations from strains HB101(pPIL110-54) (*foc*<sup>+</sup>) (lanes A), HB101(pANN801-C25) (*sfa foc*) (lanes B), HB101(pANN801-E4) (*foc sfa*) (lanes C), and HB101(pANN801-13) (*sfa*<sup>+</sup>) (lanes D). The fimbrial proteins were probed with the S adhesin-specific MAB A1 (blot I) and with the F1C-specific MAB F1-20025 (blot II). Kd, Kilodalton.

TABLE 3. Reaction of MAbs to recombinant *E. coli* strains expressing Sfa- or F1C-specific epitopes

Recombinant DNA	Fimbrial adhesion phenotype <sup>a</sup>	Reaction with MAb against:				
		S adhesin (A1) <sup>b</sup>	S fimbriae <sup>c</sup>		F1C fimbriae	
			F1	F2-4	M9-6 <sup>d</sup>	F1-20025 <sup>b</sup>
pANN801-13	Sfa-Fim <sup>+</sup> /Sfa-Adh <sup>+</sup>	+	+	+	-	-
pPIL110-54	F1C-Fim <sup>+</sup> /F1C-Adh <sup>-</sup>	-	+	-	+	+
pANN801-E4	F1C-Fim <sup>+</sup> /Sfa-Adh <sup>+</sup>	+	+	-	+	+
pANN801-C25	Sfa-Fim <sup>+</sup> /F1C-Adh <sup>-</sup>	-	+	+	-	-

<sup>a</sup> Fim, Production of fimbriae; Adh, adhesion to RBC; F1C fimbriae were set as Adh<sup>-</sup> (no agglutination of RBC [17, 44]).

<sup>b</sup> Western blot, see Fig. 6.

<sup>c</sup> Agglutination test.

<sup>d</sup> Electron micrograph after incubation of clones with immunogold-labeled antibodies; see Fig. 5.

adhesin molecules are coexpressed together with F1C fimbriae from the same hybrid gene cluster.

The functional relatedness of the *sfa* and *foc* gene clusters, demonstrated by the construction of hybrid DNA molecules (*cis*-complementations), was confirmed by *trans*-complementation studies. These experiments clearly show that *foc* gene products are able to express a wild-type phenotype, i.e., S-specific adherence, after cotransformation of *foc*-specific DNAs into strains which carry *sfa* plasmids with inserted Tn1000 elements (Table 2). The fact that P-specific gene products did not express the wild-type phenotype of *sfa* insertion mutants is also supported by previous observations that P and *foc* sequences are unable to complement each other (36; Van Die, unpublished results).

It is remarkable that the homology between the *sfa* and *foc* determinants is not restricted to their coding regions. In Southern hybridization experiments, DNAs isolated from F1C- and Sfa-producing strains hybridized with gene probes specific for DNA sequences located in the vicinity but outside of the *sfa* gene cluster. From these data, it is obvious that the regions flanking the *sfa* and the *foc* determinant at both sides are also homologous to each other. It was further demonstrated by Southern hybridizations that these flanking regions (regions of about 20 kb were tested, Fig. 1 and 3) are specific for strains carrying *sfa* and *foc* determinants. These sequences are not present in the chromosomes of strains that produce P or type I fimbriae but lack Sfa or F1C fimbriae.

Similar results were observed for the *E. coli* hemolysin determinant (*hly*), a gene cluster coding for the  $\alpha$ -hemolysin, another virulence factor of extraintestinal *E. coli* strains (27; J. Hacker, T. Schmoll, M. Ott, R. Marre, H. Hof, T. Jarchau, S. Knapp, I. Then, and W. Goebel, in E. Kass, ed., *Host-Parasite Interactions in Urinary Tract Infections*, in press). Hemolytic strains also carry *hly* determinants together with large flanking regions on their chromosomes. These tracts of DNA, which consist of the structural genes coding for  $\alpha$ -hemolysin and about 70 to 100 kb of "flanking" sequences, are not present on the chromosomes of nonhemolytic strains. In addition, it was shown that the *hly* gene clusters and their flanking sequences located on the chromosomes of different *E. coli* strains exhibit a high degree of homology (20; Hacker et al., in press; Bender, Ott, and Hacker, unpublished results). Therefore it can be concluded that the occurrence and specificity of pathogenicity determinants together with large tracts of flanking sequences in the chromosomes of pathogenic *E. coli* strains are not restricted to the *foc* and *sfa* gene clusters.

#### ACKNOWLEDGMENTS

We thank Han deRee (Weesp, The Netherlands) for providing us with MAb M9-6, Staffan Normark (Umea, Sweden) for sending the

plasmid pRHU845, and Bala Pillay (Würzburg) for critical reading of the manuscript. The excellent technical assistance of Heike Düvel is gratefully acknowledged.

The work was supported by the Deutsche Forschungsgemeinschaft (Ha1434/1-5).

#### LITERATURE CITED

1. Abe, C., S. Schmitz, I. Moser, G. Boulnois, N. J. High, I. Orskov, F. Orskov, B. Jann, and K. Jann. 1987. Monoclonal antibodies with fimbrial F1C, F12, F13, and F14 specificities with fimbriae from *E. coli* O4:K12:H<sup>-</sup>. *Microb. Pathogenesis* 2: 71-77.
2. Baga, M., M. Göransson, S. Normark, and B. E. Uhlin. 1985. Transcriptional activation of a Pap pilus virulence operon from uropathogenic *Escherichia coli*. *EMBO J.* 4:3887-3893.
3. Berger, H., J. Hacker, A. Juarez, C. Hughes, and W. Goebel. 1982. Cloning of the chromosomal determinants encoding hemolysin production and mannose-resistant hemagglutination in *Escherichia coli*. *J. Bacteriol.* 152:1241-1247.
4. Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7:1513-1522.
5. Buchanan, K., S. Falkow, R. A. Hull, and S. Hull. 1985. Frequency among *Enterobacteriaceae* of the DNA sequence encoding type 1 pili. *J. Bacteriol.* 162:799-803.
6. Clegg, S., S. Hull, R. Hull, and J. Pruckler. 1985. Construction and comparison of recombinant plasmids encoding type 1 fimbriae of members of the family *Enterobacteriaceae*. *Infect. Immun.* 48:275-279.
7. Clegg, S., J. Pruckler, and B. K. Purcell. 1985. Complementation analysis of recombinant plasmids encoding type 1 fimbriae of members of the family *Enterobacteriaceae*. *Infect. Immun.* 50:338-340.
8. de Ree, J. M., P. H. M. Savelkoul, P. Schwillens, and J. F. van den Bosch. 1986. Monoclonal antibodies raised against five different P-fimbriae and type 1A and 1C fimbriae, p. 117-118. In D. Lark, S. Normark, H. Wolf-Watz, and B. E. Uhlin. (ed.), *Protein-carbohydrate interactions in biological systems*. Academic Press Inc. (London), Ltd., London.
9. Duguid, J. P., I. W. Smith, G. Dempster, and P. N. Edmunds. 1955. Non-flagellar filamentous appendages ("fimbriae") and hemagglutination activity in *Bacterium coli*. *J. Pathol. Bacteriol.* 70:335-348.
10. Grinstedt, J., P. M. Bennett, S. Higginson, and M. H. Richmond. 1978. Regional preference of insertion of Tn501 and Tn802 into RPI and its derivatives. *Mol. Gen. Genet.* 166:313-320.
11. Hacker, J., S. Knapp, and W. Goebel. 1983. Spontaneous deletions and flanking regions of the chromosomally inherited hemolysin determinant of an *Escherichia coli* O6 strain. *J. Bacteriol.* 154:1146-1152.
12. Hacker, J., M. Ott, G. Schmidt, R. Hull, and W. Goebel. 1986. Molecular cloning of the F8 fimbrial antigen from *Escherichia coli*. *FEMS Microbiol. Lett.* 36:139-144.
13. Hacker, J., G. Schmidt, C. Hughes, S. Knapp, M. Marget, and W. Goebel. 1985. Cloning and characterization of genes involved in production of mannose-resistant, neuraminidase-susceptible (X) fimbriae from a uropathogenic O6:K15:H31 *Esch-*

- erichia coli* strain. *Infect. Immun.* 47:434-440.
14. Hopp, T. P., and K. R. Woods. 1981. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci. USA* 78:3824-3828.
  15. Jann, K., B. Jann, and G. Schmidt. 1981. SDS-polyacrylamide gel electrophoresis and serological analysis of pili from *Escherichia coli* from different pathogenic origin. *FEMS Microbiol. Lett.* 11:21-25.
  16. Källénus, G., R. Möllby, S. B. Svenson, J. Winberg, A. Lundblad, and S. Svensson. 1980. The p<sup>k</sup> antigen as receptor of pyelonephritic *E. coli*. *FEMS Microbiol. Lett.* 7:297-300.
  17. Klemm, P. 1985. Fimbrial adhesins. *Rev. Infect. Dis.* 7:321-340.
  18. Klemm, P., I. Orskov, and F. Orskov. 1982. F7 and type 1-like fimbriae from three *Escherichia coli* strains isolated from urinary tract infections: protein and immunological aspects. *Infect. Immun.* 36:462-468.
  19. Knapp, S., J. Hacker, I. Then, D. Müller, 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.
  20. Knapp, S., J. Hacker, T. Jarchau, and W. Goebel. 1986. Large instable regions in the chromosome affect virulence properties of a uropathogenic *Escherichia coli* O6 strain. *J. Bacteriol.* 168:22-30.
  21. Korhonen, T. K., V. Väisänen-Rhen, M. Rhen, A. Pere, J. Parkkinen, and J. Finne. 1984. *Escherichia coli* fimbriae recognizing sialyl galactosides. *J. Bacteriol.* 159:762-766.
  22. Korhonen, T. K., M. V. Valtonen, J. Parkkinen, V. Väisänen-Rhen, J. Finne, F. Orskov, I. Orskov, S. B. Svenson, and P. H. Mäkelä. 1985. Serotype, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. *Infect. Immun.* 48:486-491.
  23. Lederberg, E. M., and S. N. Cohen. 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. *J. Bacteriol.* 119:1072-1074.
  24. Lund, B., F. P. Lindberg, M. Baga, and S. Normark. 1985. Globoside-specific adhesins of uropathogenic *Escherichia coli* are encoded by similar *trans*-complementable gene clusters. *J. Bacteriol.* 162:1293-1301.
  25. Lund, B., B. I. Marklund, N. Strömberg, F. Lindberg, K. A. Karlsson, and S. Normark. 1988. Uropathogenic *Escherichia coli* can express serologically identical pili of different receptor binding specificities. *Mol. Microbiol.* 2:255-263.
  26. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
  27. Marre, R., J. Hacker, W. Henkel, and W. Goebel. 1986. Contribution of cloned virulence factors from uropathogenic *Escherichia coli* strains to nephropathogenicity in an experimental rat pyelonephritis model. *Infect. Immun.* 54:761-767.
  28. Moch, T., H. Hoschützky, J. Hacker, K.-D. Krönke, and K. Jann. 1987. Isolation and characterization of the  $\alpha$ -sialyl- $\beta$ -2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 84:3462-3466.
  29. 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.
  30. Nowicki, B., J. Vuopio-Varkila, P. Viljanen, T. K. Korhonen, and P. H. Mäkelä. 1986. Fimbrial phase variation and systemic *E. coli* infection studied in the mouse peritonitis model. *Microb. Pathogenesis* 1:335-347.
  31. Orskov, I., and F. Orskov. 1983. Serology of *Escherichia coli* fimbriae. *Prog. Allergy* 33:80-105.
  32. Orskov, I., and F. Orskov. 1985. *Escherichia coli* in extraintestinal infections. *J. Hyg. Cambridge* 95:551-575.
  33. 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.
  34. Ott, M., T. Schmoll, W. Goebel, I. Van Die, and J. Hacker. 1987. Comparison of the genetic determinant coding for the S-fimbrial adhesin (*sfa*) of *Escherichia coli* to other chromosomally encoded fimbrial determinants. *Infect. Immun.* 55:1940-1943.
  35. Parkkinen, J., G. N. Rogers, T. Korhonen, W. Dahr, and J. Finne. 1986. Identification of the O-linked sialyloligosaccharides of glycophorin as the erythrocyte receptors for S-fimbriated *Escherichia coli*. *Infect. Immun.* 54:37-42.
  36. Rhen, M., V. Väisänen-Rhen, and T. K. Korhonen. 1985. Complement and regulatory interaction between two cloned fimbrial gene clusters of *Escherichia coli* strain KS71. *Mol. Gen. Genet.* 200:60-64.
  37. Rigby, P. W. J., J. M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237-251.
  38. Schmitz, S., C. Abe, I. Moser, I. Orskov, F. Orskov, B. Jann, and K. Jann. 1986. Monoclonal antibodies against the nonhemagglutinating fimbrial antigen 1C (pseudotype 1) of *Escherichia coli*. *Infect. Immun.* 51:54-59.
  39. Schmoll, T., J. Hacker, and W. Goebel. 1987. Nucleotide sequence of the *sfaA* gene coding for the S fimbrial protein subunit of *Escherichia coli*. *FEMS Microbiol. Lett.* 41:229-235.
  40. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
  41. Svanborg-Eden, C., L. Hagberg, R. Hull, S. Hull, H. Leffler, H. Lomberg, and B. Nilsson. 1986. Bacterial attachment to glycoconjugate receptors: uropathogenic *Escherichia coli*, p. 135-142. In D. Lark, S. Normark, H. Wolf-Watz, and B. E. Uhlin (ed.), Protein-carbohydrate interactions in biological systems. Academic Press, Inc. (London) Ltd., London.
  42. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76:4350-4354.
  43. Van Die, I., G. Spierings, I. Van Megen, E. Zuidweg, W. Hoekstra, and H. Bergmans. 1985. Cloning and genetic organization of the gene cluster encoding F7<sub>1</sub> fimbriae of a uropathogenic *Escherichia coli* and comparison with the F7<sub>2</sub> gene cluster. *FEMS Microbiol. Lett.* 28:329-334.
  44. Van Die, I., R. Van Geffen, W. Hoekstra, and H. Bergmans. 1985. Type 1C fimbriae of a uropathogenic *Escherichia coli* strain: cloning and characterization of the genes involved in the expression of the 1C antigen and nucleotide sequence of the subunit gene. *Gene* 34:187-196.
  45. Van Die, I., I. Van Megen, E. Zuidweg, W. Hoekstra, H. De Ree, H. Van den Bosch, and H. Bergmans. 1986. Functional relationship among gene clusters encoding F7<sub>1</sub>, F7<sub>2</sub>, F9, and F11 fimbriae of human uropathogenic *Escherichia coli*. *J. Bacteriol.* 167:407-410.
  46. Wevers, P., R. Picken, G. Schmidt, B. Jann, K. Jann, J. R. Golecki, and M. Kist. 1980. Characterization of pili associated with *Escherichia coli* O18ac. *Infect. Immun.* 29:685-691.