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# Molecular cloning of the F8 fimbrial antigen from *Escherichia coli*

(*Escherichia coli*; antigen; F8 fimbriae; gene cloning)

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## 1. SUMMARY

The genetic determinant coding for the P-specific F8 fimbriae was cloned from the chromosome of the *Escherichia coli* wild-type strain 2980 (O18:K5:H5:F1C, F8). The F8 determinant was further subcloned into the *Pst*I site of pBR322 and a restriction map was established. In a Southern hybridization experiment identity between the chromosomally encoded F8 determinant of 2980 and its cloned counterpart was demonstrated. The cloned F8 fimbriae and those of the wild type strain consist of a protein subunit of nearly 20 kDa. F8 fimbriated strains were agglutinated by an F8 polyclonal antiserum, caused mannose-resistant hemagglutination and attached to human uroepithelial cells. The cloned F8 determinant was well expressed in a variety of host strains.

## 2. INTRODUCTION

*E. coli* strains cause more than three-quarters of all urinary tract infections (UTI). Several viru-

lence factors such as adhesins, O- and K-antigens, serum resistance factors and hemolysins are involved in the pathogenesis of uropathogenic strains [1,2]. The adhesive capacity of *E. coli* UTI strains is mediated by specific cell appendages called fimbriae or pili [3,4].

Most of the fimbriae from pathogenic *E. coli* strains are able to agglutinate erythrocytes in a mannose-resistant manner (mannose-resistant hemagglutination, Mrh). These Mrh fimbriae can be subdivided into P, S, M, and X fimbriae on the basis of their receptor specificities [4,5]. S fimbriae are found predominantly among *E. coli* strains which cause newborn meningitis [5] and P fimbriae among uropathogenic isolates [2,6]. P fimbriae can be divided into serologically distinct groups, termed F7–F13, but all recognize the  $\alpha$ -D-Gal-(1–4)- $\beta$ -D-Gal region of globotetraosylceramide and trihexosylceramide, which are antigens of the human blood group P system [7]. Some of these specific F-antigens have been cloned very recently [8–13] and one of them, the F13 fimbria (also called *pap*), has been studied in detail [14]. Here we describe the molecular cloning and characterization of the F8 fimbrial antigen from an O18:K5:H5:F1C, F8 wild-type isolate 2980.

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### 3. MATERIALS AND METHODS

#### 3.1. Bacterial strains, plasmids and growth media

The bacteria and plasmids used are listed in Table 1. *E. coli* strain 2980 is a wild-type strain of fecal origin of serotype O18ac:K5:F1C:F8 ([15,16], Bockemühl, personal communication). The non-fimbriated strains 536-21 (*E. coli* O6) [17] and SF1668 (*S. typhimurium*, *galE*<sup>-</sup> mutant) were used for wild-type transformation. *E. coli* K-12 strain HB101 was used as recipient in cloning procedures. Plasmid pHC79 is a cosmid cloning vector [18], and pBR322 was used for subcloning experiments.

Bacteria were cultivated in L-broth or on L-agar plates. Selective pressure against loss of plasmids was imposed by adding 50 µg/ml ampicillin or 20 µg/ml chloramphenicol.

#### 3.2. Recombinant DNA techniques and enzymes

Chromosomal and plasmid DNA were isolated as described [17,19]. Large 30-kb chromosomal *Sau*3A fragments were ligated into the *Bam*HI site of cosmid vector pHC79 and transduced into HB101 with the help of the cosmid packaging system [18], and subcloning was done into pBR322. Isolation and purification of DNA fragments from agarose gels was performed by electroelution [19]. Restriction enzymes and T4 ligase were purchased from New England Biolabs, Beveton, MA, U.S.A.

*E. coli* and *S. typhimurium* strains were transformed with the recombinant DNAs by a modified CaCl<sub>2</sub>-procedure [20].

#### 3.3. Nick translation, Southern hybridization and autoradiography

The DNA was treated with restriction enzymes and the resulting fragments were separated by agarose gel electrophoresis using 0.7–1% gels [17]. The transfer of DNA fragments from agarose gels to nitrocellulose filters, washing and autoradiography were performed as described by Southern [21]. Stringent conditions were used for the washing procedure. DNA fragments were labeled by nick translation with a mixture of all 4 α-<sup>32</sup>P-labeled dNTPs and purified by ethanol precipitation [22].

#### 3.4. Isolation of fimbriae and polyacrylamide gel electrophoresis (PAGE)

Fimbriae were isolated from bacteria grown on plates as described [15]. After disintegration of the fimbriae by boiling for 5 min in 10 mM Tris-HCl (pH 7.8) containing (in 1 ml) 4% (w/v) sodium dodecyl sulfate (SDS), 0.01 ml mercaptoethanol, 0.2 ml glycerol, and 0.002% (w/v) bromphenol blue, the samples (5 µg protein) were run on slab gels as described previously [16].

#### 3.5. Characterization of fimbriated strains

Wild-type bacteria and *E. coli* clones which

Table 1

Agglutination and adhesion properties of *E. coli* strain 2980 and clones carrying various plasmid DNAs

<i>E. coli</i> strain	Recombinant plasmid	Plasmid markers	Agglutination with			Adherence to uroepithelial cells <sup>c</sup>
			Red blood cells (MR) <sup>a</sup>	̄-Erythrocytes <sup>b</sup>	Anti-F8 serum	
2980	-	-	+++	-	++	20.2
HB101	-	-	-	-	-	1.8
HB101	pANN920	Ap <sup>R</sup> , F8	++++	-	++++	11.1
HB101	pANN921	Tc <sup>R</sup> , F8	++++	-	++++	13.5
536-21	-	-	-	-	-	2.2
536-21	pANN921	Tc <sup>R</sup> , F8	++++	-	+++	10.5
SF1572	-	-	-	-	-	nt
SF1572	pANN921	Tc <sup>R</sup> , F8	++++	-	+++	nt

<sup>a</sup> Mannose-resistant hemagglutination with human and monkey erythrocytes.

<sup>b</sup> Lack of hemagglutination with human ̄ erythrocytes indicate the presence of P-fimbriae. Hemagglutination of ̄ erythrocytes was observed with strain 536, carrying the S-fimbrial antigen [23].

<sup>c</sup> Number of attached bacteria/epithelial cell.

express P fimbriae agglutinated human or African green monkey erythrocytes in the presence of mannose, while human erythrocytes lacking the P antigens or ( $\bar{p}$  erythrocytes) could not be agglutinated. The S-fimbriated strain 536 [17] was used as a positive control for  $\bar{p}$  erythrocytes. The erythrocytes were obtained either locally (human) or from Flow Laboratories, Bonn, F.R.G. (monkey). For adhesion tests, uroepithelial cells were obtained from the fresh morning urine of a healthy subject. The adhesion test was performed as described by Svanborg-Edén et al. [3]. As demonstrated for HB101 (Table 1), the vector plasmids pHC79 and pBR322 had no effect in the hemagglutination and adhesion tests.

### 3.6. Preparation of fimbriae-specific antiserum

F8 fimbriae antiserum was prepared by injection of HB101 [pANN921] cells into rabbits as described [15,16]. HB101-specific antibodies were removed from the serum by adsorption with this strain.

## 4. RESULTS

### 4.1. Molecular cloning of the F8 fimbriae determinant

Chromosomal DNA was isolated from the O18:K5:H5 strain 2980 carrying the F8 antigen. After cleavage with *Sau3A* and a packaging procedure, 600 *E. coli* K-12 colonies were screened for the presence of the F8 antigen by hemagglutination. One recombinant clone harboring a cosmid (designated as pANN920) was found to exhibit Mrh. The presence of the F8 fimbriae genes on the cloned DNA was further confirmed by the isolation of fimbriae and by subsequent SDS-PAGE (Fig. 1), by electron microscopy (results not shown) and by the use of antibodies, directed against the F8 antigen (Table 1) (Moser et al., submitted). After transformation of pANN920 DNA into *E. coli* K-12, fimbriae were visible on the normally non-fimbriated recipient cell, indicating the presence of the F8-coding genes on this particular cosmid DNA (Table 1).

pANN920 DNA was further partially cleaved with the restriction enzyme *Pst*I, and suitable

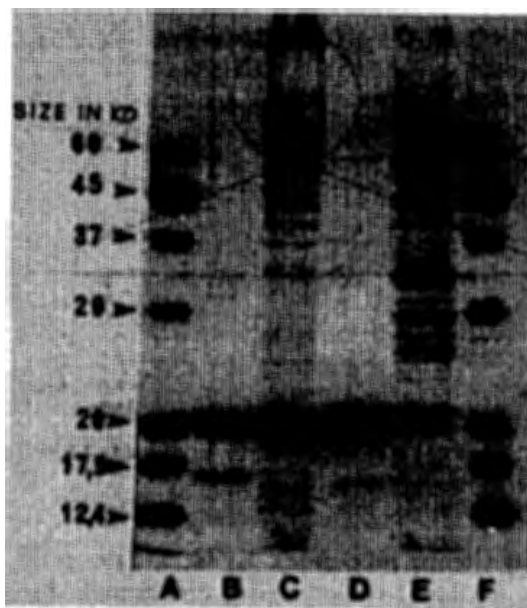


Fig. 1. SDS-PAGE of fimbrial subunits from different *E. coli* strains. Approx. 5  $\mu$ g fimbriae were mixed with 20  $\mu$ g buffer. Lanes: (A)  $M_r$  marker proteins; (B) 2980; (C) HB101 [pANN920]; (D) HB101 [pANN921]; (E) HB101; (F)  $M_r$  marker proteins.

fragments were ligated into the *Pst*I site of pBR322. One recombinant plasmid, pANN921, which had lost ampicillin resistance, still expressed resistance to tetracycline and conferred P-specific hemagglutination and fimbria formation on HB101 (Table 1).

### 4.2. Restriction map of pANN921

The recombinant plasmid pANN921 consisted of the vector pBR322 and a chromosomal insert of almost 12 kb. As shown in Fig. 2, the insert DNA, besides 2 flanking *Pst*I sites, shows 6 other internal *Pst*I recognition sequences. The physical map of pANN921 was further determined from digestion and suitable double digestions with 10 different restriction enzymes.

### 4.3. Southern hybridization

The cosmid cloning and subcloning experiments of the F8 fimbriae determinant were achieved with partially digested DNA, which could imply a rearrangement of some DNA fragments

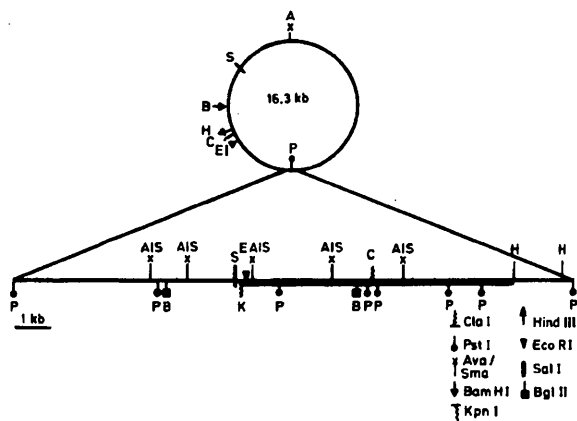


Fig. 2. Restriction map of the recombinant plasmid pANN921, coding for the F8 antigen.

during cloning procedures. To exclude this possibility for the F8-coding region, *Pst*I-cleaved chromosomal DNA of strain 2980 and pANN921 plasmid DNA were hybridized against an  $\alpha$ - $^{32}$ P-labeled probe of the F8 determinant. As a probe, a 6.0-kb *Kpn*I-*Hind*III fragment was chosen. As judged from the restriction maps of other cloned F determinants, which share sequence homology [8-14], this fragment should carry most of the internal F8 coding region. As shown in Fig. 3, identical *Pst*I fragments were obtained from the chromosomal and the plasmid DNA. Two *Pst*I fragments, of 2.6 kb and 1.9 kb, represented the flanking regions of the *Kpn*I-*Hind*III fragment. 4 other fragments were also visible in the autoradiogram (3 *Pst*I fragments of 1.9 kb, 1.9 kb and 1.8 kb in size are visible as a broad single band in Fig. 3). Thus, the cloned F8 determinant has the same arrangement as the one in the chromosome.

#### 4.4. Characterization of the F8 fimbriae

After purification and dissociation of the fimbriae with SDS, 20-kDa protein subunits were obtained in PAGE from the wild-type strain 2980 and from *E. coli* K-12 clones harboring the recombinant DNAs pANN920 and pANN921 (Fig. 1). A second band of 16.5 kDa, visible in preparations of *E. coli* 2980, may represent the F1C fimbriae of this strain [16].

F8-specific rabbit antiserum was obtained by absorption with the plasmid-free *E. coli* K-12

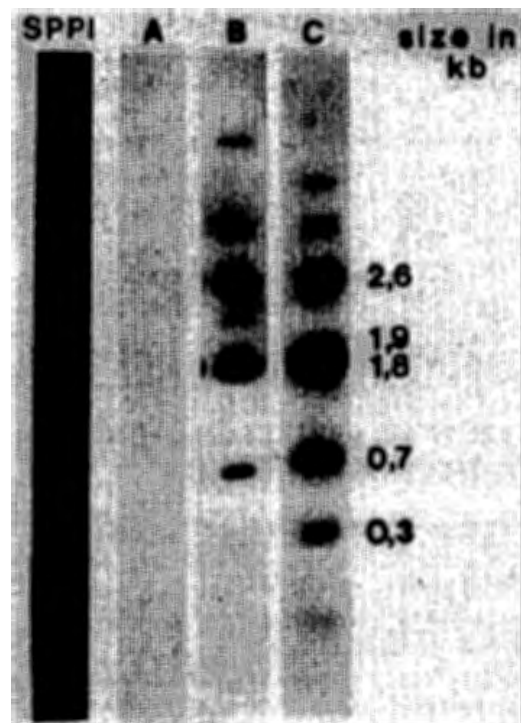


Fig. 3. Hybridization patterns of *Pst*I-cleaved chromosomal DNA from *E. coli* K-12 HB101 (Lane A), 2980 (Lane B) and plasmid DNA from HB101 [pANN921] (Lane C). As hybridization probe, a nick-translated  $\alpha$ - $^{32}$ P-labeled 6.0-kb *Kpn*I-*Hind*III fragment of pANN921 was used. Cleaved SPPI-DNA was used as size marker.

strain HB101 of an antiserum raised against the F8-fimbriated *E. coli* HB101 pANN921 clone. As indicated in Table 1, wild-type strain 2980 and strains carrying the cloned F8 determinant reacted strongly with this serum, while HB101 showed no reaction. These tests were further confirmed by the use of monoclonal antibodies against the F8 antigen (Moser et al., submitted), confirming identity between the wild-type and the cloned F8 fimbriae.

The F8-specific DNA pANN921 was further transformed into the non-fimbriated wild-type *E. coli* 06 strain 536-21 [17] and a *S. typhimurium galE*-mutant. In both cases fimbriae were well expressed and reacted with erythrocytes and with specific antiserum (Table 1).

Additionally, attachment of bacteria to human uroepithelial cells was only visible after introduc-

tion of the cloned fimbriae determinants into the bacteria, indicating that the F8 antigen encoded by the recombinant plasmids is functioning.

## 5. DISCUSSION

In this report the molecular cloning of the genes coding for the F8 fimbriae antigen is described. After a cosmid cloning procedure, a 12-kb piece of DNA was inserted into the *Pst*I site of pBR322. This chromosomal DNA insert conferred P-specific mannose-resistant hemagglutination and F8 fimbriae formation.

The size of the coding regions of other chromosomally encoded fimbriae ranges between approx. 5.0 and 8.5 kb [8-14,23]. Therefore, it is suggested that the region coding for F8 fimbriae is smaller than the 12-kb segment inserted into pBR322. On the basis of the restriction sites, no similarities were revealed between the cloned S-fimbrial antigen (*sfa*) determinant and the F8 genes [23]. Some restriction sites, however, such as a *Cla*I site and a *Kpn*I site, seem to be conserved in different cloned P-fimbrial determinants coding for F8, F9, F11, F7, F13 or F 'Clegg' [8-14]. It therefore appears that a 6.0-kb DNA fragment which resulted from a *Kpn*I-*Hind*III double cleavage of pANN921 carried most of the F8 coding region. Thus such a fragment was used as radioactively labeled probe in Southern blots (Fig. 3). The identity of the 6 *Pst*I fragments forming the chromosomal and the cloned F8 plasmid DNA rules out the possibility that DNA rearrangement has occurred following the cloning procedure.

The size of the F8 fimbrial subunit was found to be nearly 20 kd, which is in good agreement with the  $M_r$  value of 19.7 kd published by Moser et al. (submitted for publication) and is within the range of 15-29 kd that has been described for other fimbriae [8-14]. No differences in size could be seen between F8 fimbriae isolated from the wild-type isolate and from the K-12 clones harboring the cloned F8 DNA. A large percentage of *E. coli* O18:K5 wild-type isolates exhibit the F8 antigen as demonstrated by different serological techniques [2,24] (Moser et al., submitted). As indicated by Achtman and co-workers, all these

O18:K5:F8 strains exhibit the outer membrane pattern (Omp) 11 and the flagellar antigen 5; this has also been demonstrated in the case of strain 2980 (Bockemühl, personal communication). It would be interesting to determine by means of genetical or serological methods whether other O18:K5 variants showing the membrane pattern 6 and 18 and (in the case of Omp18) the flagellar antigen 7 [2,25] also produce the F8 antigen or P fimbriae with distinguishable serological and genetic properties.

As can be seen from Table I, the F8-encoding recombinant plasmid pANN921 could be transformed into various wild-type hosts, such as an *E. coli* O6 strain and an *S. typhimurium galE*<sup>-</sup> strain. Thus, unlike some adhesins from intestinal *E. coli* strains [26], the expression of the F8 antigen does not seem to interfere with these host bacteria. The introduction of the F8 determinant into such strains offers possibilities for the construction or novel live vaccines, as already suggested for the *E. coli* K88 adhesin [27].

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