Characterization of DNA Fragments Encoding Fimbriae of the Uropathogenic Escherichia coli Strain KS71

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Recombinant plasmids were constructed that expressed the KS71A, KS71B and KS71C fimbrial antigens of the pyelonephritogenic Escherichia coli strain KS71 (O4 : K12) in E. coli HB101. The KS71C-encoding genes were located on a 6.4 kb HindIII-XhoI fragment obtained from the recombinant cosmid pKTH145 that expresses this antigen. Spontaneous KS71C- mutants were isolated that contained a 0.8 kb insert in a specific restriction fragment of KS71C-encoding recombinant plasmids. The KS71B-encoding segment was located on a 11.5 kb deletable DNA fragment of recombinant cosmid pKTH144. A DNA fragment encoding the KS71A fimbria was obtained on a 12 kb EcoRI fragment of the recombinant cosmid expressing this antigen in E. coli HB101 and closely resembled the KS71B-encoding fragment. In the recombinant cosmid, the KS71B-expressing region was flanked by homologous DNA segments. A similar stretch of DNA was found close to the KS71A-expressing DNA region.

INTRODUCTION

Fimbriae are proteinaceous surface filaments (Brinton, 1965; Duguid et al., 1955) which mediate bacterial adhesion to both animal (Burrows et al., 1976; Duguid, 1968; Svanborg Eden et al., 1976) and plant cell surfaces (Korhonen et al., 1983). The adhesion of fimbriated bacteria to animal cells can be demonstrated by haemagglutination of erythrocytes (Källenius & Möllby, 1979), but nonhaemagglutinating fimbriae have also been described (Klemm et al., 1982; Ørskov et al., 1980). Fimbriae that mediate adhesion to uroepithelial cells are thought to contribute to the virulence of pyelonephritogenic Escherichia coli (Källenius et al., 1981; Väisänen et al., 1981). Such fimbriae have been termed P-fimbriae (Korhonen et al., 1982). Their receptor on both human erythrocytes and uroepithelial cells is the α-D-Gal-(1→4)-β-D-Gal disaccharide moiety of the P-blood-group-specific glycosphingolipids (Källenius et al., 1981; Korhonen et al., 1982).

Our model strain, the pyelonephritogenic E. coli strain KS71 (O4 : K12), expresses four types of fimbriae (Rhen et al., 1983a, d). These are the mannose-binding type-1 fimbriae (KS71D), two variants of P-fimbriae (KS71A and KS71B) that are immunologically cross-reactive, and the non-haemagglutinating KS71C fimbriae (Rhen et al., 1983a, b). The fimbrial composition of strain KS71 is similar to that of E. coli C1212, the model strain for F7 fimbrial antigens (Ørskov et al., 1980). An intriguing feature of E. coli KS71 fimbriae is the occurrence of rapid phase variation between the different fimbrial types (Rhen et al., 1983c). P and KS71C fimbriae are not expressed on the same cells; isolated subpopulations having either P or KS71C fimbriae soon express the other type when recultured.

Gene clusters encoding mannose-resistant fimbriae of three uropathogenic E. coli strains have been cloned and characterized (Clegg & Pierce, 1983; Normark et al., 1983; Van Die et al., 1984). We have recently cloned structural genes for KS71A, KS71B and KS71C fimbriae separately into a non-fimbriated E. coli K12 derivative (Rhen et al., 1983b). This communication describes the comparison and some characteristics of these genes.
Bacterial strains and cultivation. E. coli strains AM1727 (RecA-, Van Die et al., 1983) and HB101 (Leu- Pro- Lac- HsdS- Gal- StrA- Thi- RecA-) were used as recipients. All strains were grown for 16-18 h at 37°C in Luria broth or on Luria agar supplemented with ampicillin, 100 μg ml⁻¹ (Wyeth Laboratories, USA); chloramphenicol, 20 μg ml⁻¹ (Sigma) or tetracycline, 15 μg ml⁻¹ (Sigma).

Cloning procedures and restriction mapping. Restriction endonucleases and T4 DNA ligase were obtained from Boehringer Mannheim and Amersham. The enzymes were used under conditions described by Maniatis et al. (1982). The cloning vectors pACYC184 and pBR322 were described by Chang & Cohen (1978) and Bolivar et al. (1977), respectively. Ligated DNA and isolated plasmid DNA was introduced into recipients by transformation of CaCl₂-treated cells (Mandel & Higa, 1970). Plasmid DNA was isolated from cell lysates by ethidium bromide/caesium chloride equilibrium gradient centrifugation (Clewell, 1972; Maniatis et al., 1982). For screening purposes the plasmids were isolated by the alkaline lysis method (Birnboim & Doly, 1979). For restriction mapping, fragments obtained by single, double or triple digests of plasmid DNA were analysed in gels containing between 0.5% and 1% agarose, depending on the size of fragments separated.

DNA hybridization tests. Hybridization (Southern, 1975) was performed according to Maniatis et al. (1982). Radioactive probes were synthesized by nick translation (Rigby et al., 1977) in the presence of [³²P]dCTP (Amersham). Radioactive bands were detected with Kodak X-Omat films.

Immunological methods. Antisera against KS71A, KS71C and KS71ABC fimbriae of E. coli KS71 and type 1 fimbriae from E. coli 2131 were those described previously (Korhonen et al., 1982; Rhen et al., 1983a). Rabbit anti-KS71B serum was obtained by immunizing New Zealand rabbits (Korhonen et al., 1982; Rhen et al., 1983b). Specific antisera against KS71A and KS71B fimbriae were obtained by absorbing anti-KS71A serum with KS71B-expressing recombinant E. coli cells (EH825) and anti-KS71B serum with KS71A-expressing recombinant E. coli cells (EH824).

Colony blotting, employing anti-KS71C at a dilution of 1/100 and ¹²⁵I-labelled protein A (Pharmacia), was performed essentially as described by Bittner et al. (1980) and Väisänen-Rhen (1984).

Agglutination tests and electron microscopy. Bacterial agglutination with antisera and haemagglutination of human OP1 and Op erythrocytes in the presence of 5% (w/v) methyl a-mannopyranoside, as well as electron microscopy of negatively stained bacteria, was performed according to Rhen et al. (1983a, b).

RESULTS AND DISCUSSION

Subcloning and characterization of a DNA fragment encoding KS71C fimbriae

The 47 kb recombinant cosmid pKTH145 encoding KS71C fimbriae was digested with endonuclease SalI followed by ligation at a low DNA concentration. The plasmid obtained, pKTH3001 (Fig. 1), expressed KS71C fimbriae in E. coli HB101 (Table 1) and contained, in

Table 1. Agglutination properties of recombinant E. coli strains

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Plasmid carried*</th>
<th>anti-KS71A</th>
<th>anti-KS71B</th>
<th>anti-KS71C</th>
<th>Haemagglutination titre$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB101</td>
<td>pKTH145, pKTH3001-3004</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>5120-10240</td>
<td>0</td>
</tr>
<tr>
<td>HB101</td>
<td>pKTH3005-3008, pKTH3012</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>0</td>
</tr>
<tr>
<td>HB101</td>
<td>pKTH144, pKTH3010</td>
<td>640</td>
<td>&lt;10</td>
<td>2560 (5120)</td>
<td>10</td>
</tr>
<tr>
<td>HB101</td>
<td>pKTH3011, pKTH3019</td>
<td>&lt;10 (640)</td>
<td>2560 (5120)</td>
<td>&lt;10</td>
<td>32-64§</td>
</tr>
<tr>
<td>HB101</td>
<td>pKTH3009, pKTH3013</td>
<td>640</td>
<td>&lt;10</td>
<td>2560 (5120)</td>
<td>10</td>
</tr>
<tr>
<td>AM1727</td>
<td>pKTH3011, pKTH3019</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>0</td>
</tr>
<tr>
<td>HB101</td>
<td>pKTH143, pKTH3020</td>
<td>2560 (5120)</td>
<td>&lt;10 (80)</td>
<td>&lt;10</td>
<td>32-64§</td>
</tr>
<tr>
<td>HB101</td>
<td>pKTH3021, pKTH3022</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>0</td>
</tr>
</tbody>
</table>

* Plasmids causing identical agglutination reactions in HB101 or AM1727 are grouped.
† Agglutination titre with anti-type-1 serum was <10, with pKTH175, pKTH3001-3007 <10-20.
‡ With cross-absorbed antisera: titres in parenthesis were obtained with unabsorbed antisera.
§ With human OP, erythrocytes: none of the strains agglutinated OP erythrocytes.
¶ Cultures from strongly haemagglutinating colonies.
∫ Cultures from weakly haemagglutinating colonies.
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Fig. 1. Restriction endonuclease maps of pKTH3001 and of recombinant plasmids derived from it. The thick lines represent DNA sequences of the cloning vectors (for pH7C7; for pBR322; for pACYC184), whereas the thin line represents insert DNA. There was a 0.8 kb insert, marked with a dotted line, in pKTH3012, otherwise the maps of pKTH3003 and pKTH3012 appeared identical. The bar is 1 kb.

addition to the cosmid vector, 14.4 kb of insert DNA. HindIII fragments of pKTH3001 were then ligated into the HindIII sites of pACYC184 and pBR322. The KS71C-expressing recombinant plasmids obtained, pKTH3002 and pKTH3003, contained the 7.2 kb HindIII fragment of pKTH3001 in the same orientation in respect to the tetracycline resistance gene of pACYC184 and pBR22, respectively.

In order to estimate the size of the DNA fragment that encoded KS71C fimbriae in pKTH3003, several deletion mutants were constructed (Fig. 1). The plasmid pKTH3004 contained a 0.5 kb deletion obtained by SalI–XhoI digestion, whereas the plasmids pKTH3005 and pKTH3006 were produced, respectively, by deleting a 1.9 kb ClaI and a 3.5 kb EcoRI fragment of pKTH3003. pKTH3007 was obtained by ligating the EcoRI–XhoI digest of pKTH3003 with EcoRI-treated pACYC184 DNA. Of these plasmids, only pKTH3004 produced KS71C fimbriae on the cell surface in E. coli HB101 (Table 1). The structural gene for the KS71C fimbrilllin (17 kDa) would need less than 0.6 kb of DNA, which clearly goes below the size (6.4 kb) of the insert in pKTH3004. The loci encoding the K88ab fimbrial antigen of enterotoxigenic E. coli (about 6 kb in size) and mannose-resistant fimbriae of uropathogenic E. coli (about 9 kb in size) contained, in addition to the structural one, genes that encoded assembly and transport functions of the fimbrilllin (Mooi et al., 1981; Normark et al., 1983). The region encoding KS71C fimbriae may also contain accessory genes needed for the expression of the fimbral antigen.

Isolation of spontaneous KS71C− mutants

Upon transformation of pKTH3002 or pKTH3003 into E. coli HB101, recipients were found to be variable in colony morphology. After overnight growth on agar plates, KS71C-positive colonies were opalescent, about 1 mm in diameter, and reacted strongly with anti-KS71C serum (Table 1). Larger and more translucent colonies were also observed occasionally. Such colonies were not agglutinated by anti-KS71C serum (Table 1). Cells of small colonies appeared fimbriated in the electron microscope, whereas cells of large colonies appeared non-fimbriated. The proportion of the larger colonies was $3 \times 10^{-2}$ to $6 \times 10^{-2}$, depending on the pKTH3002 or pKTH3003 batch used for transformation.
Plasmids were isolated from recultures of 12 independent KS71C− colonies obtained upon transforming E. coli HB101 with various batches of pKTH3002 DNA. These plasmid preparations were then treated with ClaI and HpaI and the restriction fragments were compared with those of pKTH3002 in agarose gels (Fig. 2, lanes e and f). The restriction fragments exhibited identical patterns in all the plasmid preparations from KS71C− colonies. One such plasmid, pKTH3008, was selected for further investigation. A 0.8 kb insert was observed in a specific ClaI–HpaI fragment when the restriction maps of pKTH3008 and pKTH3002 were being compared (Fig. 1). Identical insertions, leading to the KS71C− phenotype, were also observed in transformation experiments with pKTH3003 (mutated plasmids obtained from pKTH3003 were termed pKTH3012). The origin and function of this insert is unknown. It may originate from a duplication or it may be an insertion element that originates from the chromosome.

Next, pKTH3008 DNA was transformed into E. coli HB101. None of the 10^4 transformants tested reacted positively with anti-KS71C serum in the immune blotting assay.

**Variation in the expression of KS71B fimbriae in EH825 and EH864**

In this study three types of haemagglutination were identified for EH825, a recombinant cosmid E. coli strain that produces KS71B fimbriae (Rhen et al., 1983a). Upon reculturing, EH825 formed colonies that caused an immediate, strong haemagglutination of human P1 erythrocytes (Table 1). When pKTH144, the recombinant cosmid of EH825, was transformed into E. coli HB101 only weakly haemagglutinating (36) and non-haemagglutinating (80) colonies were obtained among the 116 colonies tested (Table 1).

Recombinant cosmids were isolated from 50 independent weakly haemagglutinating and from 50 independent nonhaemagglutinating pKTH144 transformants and subjected to BamHI–ClaI restriction fingerprinting. All cosmids of the weakly haemagglutinating isolates appeared identical. However, a deletion of about 12 kb was observed in cosmids of the nonhaemagglutinating colonies and all deletions appeared identical (Fig. 2, lanes a–d). The deleted cosmid was termed pKTH3009.
Restriction maps of pKTH144 from strongly and weakly haemagglutinating EH825 appeared identical and consisted of three BamHI fragments (BI, BII and BIII; Fig. 3). BI, which appeared deleted in pKTH3009, was cloned into the BamHI site of pACYC184 from pKTH144 of weakly haemagglutinating EH825. This pACYC184 derivative was termed pKTH3011. EH866, the E. coli HB101 strain carrying pKTH3011, was strongly haemagglutinating (Table 1). Weakly haemagglutinating EH866 colonies were obtained when pKTH3011 was transformed into E. coli HB101. In contrast to the pKTH144 transformants, strongly haemagglutinating colonies were occasionally found among the pKTH3011 transformants (4/100). No differences were observed in the restriction maps when plasmids isolated from strongly haemagglutinating colonies were compared to those isolated from weakly haemagglutinating colonies, and plasmids isolated from both types of colonies gave weakly and strongly haemagglutinating colonies upon transformation (mean ratio of strongly/weakly = 3/100). The orientation of the BI fragment in respect to the tetracycline resistance gene had no effect on the variable KS71B-expression. In E. coli AM1727, pKTH3011 produced a strong and constant expression of KS71B-fimbriae (Table 1).

**The DNA fragment encoding KS71B-fimbriae is flanked by homologous DNA sequences**

The BII fragments of pKTH144 and pKTH3009 were cloned separately into the BamHI site of pACYC184 and the new recombinant plasmids termed pKTH3013 and pKTH3014, respectively (Fig. 3). BamHI–SalI fragments lining the deletable DNA segment of pKTH144 were then subcloned. The 1.8 kb BamHI–SalI fragment of pKTH3011 was further subcloned in pACYC184, and the 1.8 kb BamHI–SalI fragment of pKTH3013 and the 1.2 kb BamHI–SalI fragment of pKTH3014 were subcloned separately in pBR322. The new plasmids were termed pKTH3015, pKTH3016 and pKTH3017, respectively.
Fig. 4. Ethidium bromide stained agarose gels and Southern blot analysis of plasmids derived from pKTH144. \(\text{BamHI-SalI} \) digests of pKTH3011 (a) and pKTH3013 (b) were hybridized with a probe prepared from pKTH3015 (e and f, respectively), with probes prepared from pKTH3016 (i and j, respectively) and pACYC184 (m and n, respectively). \(\text{BamHI-SalI-EcoRI} \) digests of pKTH3013 (c) and pKTH3014 (d) were hybridized with probes prepared from pKTH3015 (g and h, respectively), pKTH3016 (lanes k and l, respectively) and pACYC184 (lanes o and p, respectively). \(\text{SalI-ClaI-HindIII} \) digests of pKTH3023 (a derivative of pKTH3011 with the 1.8 kb \(\text{BamHI-SalI} \) fragment of BI deleted) and pBR322 (internal molecular weight standard) were hybridized with probes prepared from pBR322 (lane q) and pKTH3016 (lane r). Control experiments with pBR322 as probe gave the same hybridization profiles as pACYC184 did. The sizes of the DNA fragments (in kb) are indicated at the edges of the pictures.

Radioactive probes were synthesized from pKTH3015, from pKTH3016 and from corresponding cloning vectors. The insert of pKTH3015 hybridized with the 2.4 kb \(\text{EcorRI-SalI} \) fragment of pKTH3013 and pKTH3014 (Fig. 4, lanes b, c, d and f, g, h) and with a homologous segment in pKTH3011 (Fig. 4, lanes a and e). Using a pKTH3016 probe hybridization was observed with the 9.5 kb \(\text{BamHI-SalI} \) fragment of pKTH3011 (Fig. 4, lanes a and i) and with the 1.8 kb and 1.2 kb \(\text{BamHI-SalI} \) fragments of pKTH3013 and pKTH3014, respectively (Fig.
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4, lanes a, c, d and i, k, l). Recombinant plasmid pKTH3023 was produced by deleting the 1.5 kb SalI fragment of pKTH3011. A 0.3 kb SalI–HindIII fragment of pKTH3023 hybridized with the pKTH3016 probe (Fig. 4, lanes q and r).

No cross-hybridization was observed between inserts of pKTH3015 and pKTH3016 (Fig. 5). Probes prepared from both of these plasmids hybridized with the 1.2 kb BamHI–SalI fragment of pKTH3017 (Fig. 4, lanes h and i) indicating that it was a hybrid fragment produced during the deletion. Only the 0.8 kb BamHI–SmaI fragment of pKTH3017 showed hybridization with pKTH3015 (Fig. 5, lanes i and n) whereas the 0.4 kb SmaI–SalI fragment, but not the BamHI–SmaI one of pKTH3017, hybridized with the insert in pKTH3016 (Fig. 5, lanes q and r). Restriction fragments corresponding to the cross-hybridizing fragments of the insert in pKTH3017 were present in the restriction site sequences BamHI–SmaI–AccI–SalI and BamHI–PstI–SmaI–SalI of the inserts in pKTH3015 and pKTH3016, respectively (Fig. 3). If one assumes that the restriction site sequence BamHI–SmaI of pKTH3015 was left intact during the deletion and that the homologous DNA in the 2.4 kb SalI–EcoRI fragment adjacent to the cloned insert of pKTH3016 in pKTH3013 was a fragment corresponding to the SmaI–SalI fragment of pKTH3015, then the deletable fragment of pKTH144 was flanked by inversely orientated DNA segments consisting of roughly the 1.2 kb restriction site sequence SmaI–AccI–SalI–HindIII–SmaI.

The precise role of the homologous regions lining the region expressing KS71B fimbriae in pKTH144 remains unclear, although they may be involved in the deletion of fimbrial genes. Recently Hacker et al. (1983) and Low et al. (1984) described flanking sequences around haemolysin genes of uropathogenic E. coli. In strains of serogroups O4 and O6 the genes for haemolysin and fimbriae production seemed clustered and it was suggested that haemolysin genes could have been received by a transpositional event (Low et al., 1984). Possibly, P fimbriae genes have also evolved by transposition.

Comparison of recombinant plasmids encoding KS71A and KS71B fimbriae

A plasmid (pKTH3020) expressing KS71A fimbriae in E. coli HB101 was obtained from the 45 kb recombinant cosmid pKTH143 by treating cosmid DNA with EcoRI followed by ligation at a low DNA concentration. In contrast to recombinant cells expressing KS71B fimbriae there was no quantitative fluctuation in the KS71A expression in E. coli HB101 carrying pKTH3020. In both pKTH3011 and pKTH3020 there was a restriction site sequence HpaI–BglII–HpaI–HindIII–HpaI, also the sites for AccI, ClaI and SmaI appeared to be similarly located within these sequences (Fig. 6). The fimbrial antigens encoded by these sequences were serologically partially cross-reactive (Table 1). The restriction site sequence SmaI–AccI–SalI–HindIII–SmaI at the left end of the insert in pKTH3011 was present at the right end of the insert in pKTH3020. These sequences corresponded to the proposed flanking sequence in pKTH144.

Four new recombinant plasmids, pKTH3018, pKTH3019, pKTH3021, pKTH3022, were constructed, of which only pKTH3019 produced haemagglutination in recipient strains (Table 1). pKTH3018 was produced by deleting the 10 kb ClaI fragment of pKTH3011 and pKTH3019 was obtained by partial HindIII deletion of pKTH3010, a pACYC184 derivative containing the BI fragment of pKTH3011 in opposite orientation in the BamHI cloning site. pKTH3021 and pKTH3022 were obtained by subcloning the BglII(2,3) fragment of pKTH3020 in pACYC184 and the BglII(1,4) fragment of pKTH3020 as such.

Using a probe prepared from pKTH3019, cross-hybridization was demonstrated with the 5.1 kb HpaI(2–5)HpaI(3), one or both of the 0.4 kb HpaI(3–4)–BglII(4), the 1.9 kb BglII(3–4)–HpaI(2) and the 1.4 kb HpaI(4–5) fragment of pKTH3020 (Fig. 7). The same probe hybridized with the 1.2 kb HindIII(2–5)–HpaI(3), one or both of the 2.5 kb HpaI(3–4)–HindIII(3) and the 0.8 kb HpaI(3–4) fragments of pKTH3020. The 1 kb HpaI(6)–EcoRI fragment did not hybridize (Fig. 7, lane c). The pKTH3019 probe did not hybridize with the KS71C-encoding insert of pKTH3002. A pKTH3021 probe hybridized with the 0.7 kb SmaI and 1.2 kb SmaI–ClaI fragments of pKTH3018 (Fig. 8, lanes a, b, c). Probes prepared from pKTH3015 and pKTH3016 hybridized with the 1.2 kb SmaI fragment of pKTH3022 (Fig. 8, lanes d, e). No such hybridization was observed with pKTH3019.
Fig. 6. Restriction endonuclease maps of pKTH3011 and pKTH3020. Recombinant plasmids pKTH3018 and pKTH3019 are deletion mutants of pKTH3011 and pKTH3010 (see text), respectively. pKTH3021 and pKTH3022 are deletion mutants of pKTH3020. The line symbols are the same as in Fig. 1. The bar is 1 kb.

Fig. 7. Ethidium bromide stained agarose gels and Southern blot analysis of restriction fragments obtained from pKTH3020. Lanes (a), (b), (c) and (d) show the HpaI–BglII, HpaI–HindIII, HpaI–EcoRI and HpaI–XhoI digests of pKTH3020. These restriction digests are in the same order in lanes (e), (f), (g), (h) and in lanes (i), (j), (k), (l). Lanes (e), (f), (g), and (h) were hybridized with a radioactive probe prepared from pKTH3019, whereas lanes (i), (j), (k) and (l) were hybridized with a radioactive probe prepared from pACYC184. Lane (m) contains the BglI–XhoI digest of pKTH3020; in lane (n) these fragments have been hybridized with the pKTH3019 probe. The sizes of the restriction fragments (in kb) are indicated at the sides of the pictures.
In this communication I have compared DNA sequences encoding three different fimbriae of one uropathogenic *E. coli* strain. The DNA sequences encoding the serologically and functionally related KS71A and KS71B fimbriae were similar, which may reflect a close evolutionary relationship. A novel finding was that the KS71B-encoding genes were located on a deletable DNA fragment flanked by homologous DNA sequences. Only one set of these sequences was recovered in the recombinant plasmid encoding KS71A fimbriae and these genes were not delectable. The DNA sequence encoding KS71C fimbriae appeared different from those encoding KS71A and KS71B fimbriae.

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**REFERENCES**


KÅLLENIUS, G. & MÖLLBY, R. (1979). Adhesion of *Escherichia coli* to human periurethral cells correlat-


