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Cloning and genetic organization of the gene cluster encoding F7₁ fimbriae of a uropathogenic *Escherichia coli* and comparison with the F7₂ gene cluster

(Recombinant DNA; mannose-resistant hemagglutination; fimbriae genetics)

Irma van Die, Gonnie Spierings, Ingrid van Megen, Elly Zuidweg, Wiel Hoekstra and Hans Bergmans

Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

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1. SUMMARY

The gene cluster coding for expression of F7₁ fimbriae of the uropathogenic *Escherichia coli* strain AD110 has been cloned by a cosmid-cloning procedure. A positive clone was further subcloned to a plasmid of 17.5 kilobases (kb), pPIL110-75. Analysis of pPIL110-75 showed that at least six genes are present encoding proteins with apparent M_r s of 75 000, 36 000, 23 000, 20 000, 17 000 and 14 000. The 20-kDa protein, encoding the F7₁ fimbrial subunit is dispensable for expression of the MRHA phenotype. Complementation experiments of mutants in the F7₂ gene cluster by gene products of the F7₁ gene cluster show that the two gene clusters are related.

2. INTRODUCTION

For uropathogenic *E. coli* strains a strong correlation has been found between virulence and the expression of fimbriae that enable the bacteria to adhere to uroepithelial cells [1]. These fimbriae

also cause mannose-resistant hemagglutination MRHA of human erythrocytes. Most (about 80%) of these MRHA fimbriae recognize the α -D-Gal-(1-4)- β -D-Gal moiety of the P blood group antigens [2–5], and are therefore called P-fimbriae. Ørskov and Ørskov [6] distinguish at least eight different serotypes among P-fimbriae and they suggest to refer to them as F-antigens.

Many uropathogenic *E. coli* strains carry several types of fimbriae on their cell surface, which raises questions about the genetic regulation and the function of these fimbriae. The uropathogenic *E. coli* strain AD110 [7–9] expresses, next to type 1 and 1C fimbriae, two types of P-fimbriae, F7₁ and F7₂. Cloning and characterization of these fimbriae should facilitate studies of the genetic organization and regulation of these fimbriae. The gene clusters coding for F7₂ fimbriae and 1C fimbriae of AD110 have recently been cloned and characterized [10–13]. Biosynthesis of fimbriae requires expression of the gene encoding the fimbrial subunit as well as expression of a number of genes involved in processing of the subunits into fimbriae. In this paper the cloning and characterization of the gene

cluster encoding F7₁ fimbriae is described. The gene cluster is compared with the gene cluster encoding F7₂ fimbriae.

3. MATERIALS AND METHODS

3.1. Bacterial strains, plasmids and recombinant DNA techniques

E. coli strains AD110, JA221 and P678-54 have been described before [10,11]. Plasmid vectors used were pJB8 [14] and pBR322 [15]. The cosmid cloning procedure and other DNA techniques have been described before [10,11]. Transformation was carried out essentially as described by Kushner [16].

3.2. Characterization of clones carrying recombinant DNA

Analysis of plasmid-encoded proteins in minicells was performed essentially as described in [11]. Hemagglutination of human erythrocytes by bacterial cells and agglutination of bacterial cells in antiserum were performed as described in [10]. Purification of fimbriae and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were carried out as described in [10].

4. RESULTS AND DISCUSSION

4.1. Cloning of the genetic determinants encoding F7₁ fimbriae

A cosmid colony bank of AD110 [10] in the *E. coli* K-12 strain JA221 was screened for the presence of clones that show MRHA of human erythrocytes. Fimbriae were isolated from a number of MRHA-positive clones and analyzed by SDS-PAGE. The F7₁ and F7₂ fimbriae are easily distinguished on the basis of their apparent M_r s of 20 000 and 17 000, respectively [10,11]. One colony expressing F7₁ fimbriae was selected. The colony harbored a plasmid (pPIL110-7) of the expected size (about 40 kb). pPIL110-7 contains two *Bam*HI restriction sites. Digestion of pPIL110-7 with *Bam*HI followed by religation and transformation into JA221 resulted into transformants still showing MRHA. These transformants harbored a plasmid (pPIL110-70) of 25 kb. The genes encoding MRHA were further subcloned to a plasmid of 17.5 kb (pPIL110-75) by deleting a *Sal*I fragment and two *Eco*RI fragments. The physical maps of pPIL110-7 and the subclones is shown in Fig. 1.

JA221 cells carrying pPIL110-75, unlike JA221 cells, were agglutinated by antiserum raised against all fimbriae of AD110, JA221 cells carrying

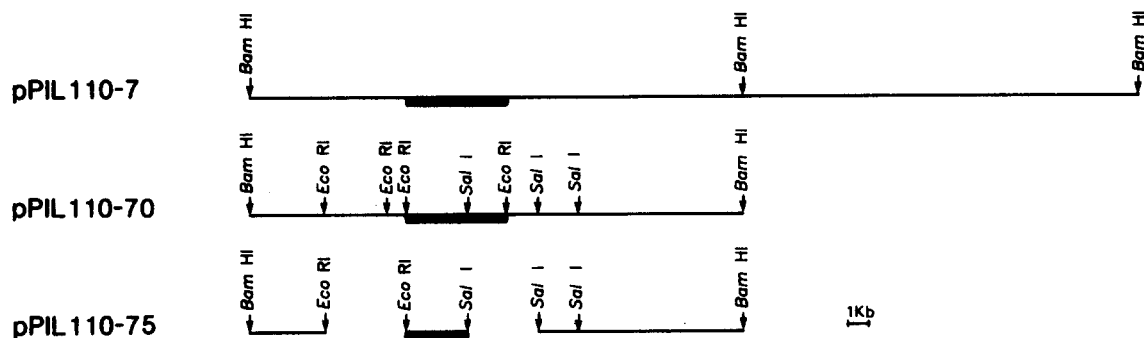


Fig. 1. Physical maps of pPIL110-7, pPIL110-70 and pPIL110-75. The black boxes represent pJB8 DNA.

pPIL110-75 showed in a MRHA test a titer of 1/128, which is stronger than the MRHA titer of AD110 (1/8) whereas JA221 did not show MRHA.

Summarizing we conclude that pPIL110-75 carries the genes responsible for the expression of F₇₁ fimbriae.

4.2. Analysis of plasmid-coded proteins in minicells

The presence of pPIL110-75 in minicells leads to the expression of proteins of 75, 40, 36, 23, 20, 17.5, 17, 15 and 14 kDa, as well as proteins expressed by the vector (Fig. 2, lane a; Table 1).

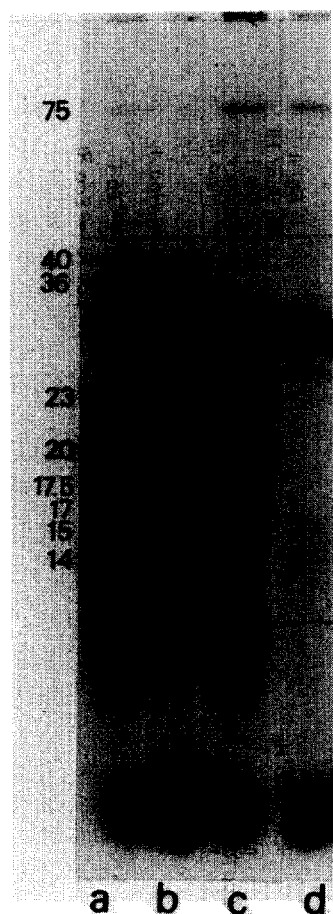


Fig. 2. Autoradiogram of ¹⁴C-labeled proteins of P678-54 minicells separated by SDS-PAGE. In lanes a–d total labeled proteins, denatured at 95°C, are shown of minicells harboring the following plasmids: (a) pPIL110-75, (b) pPIL110-751, (c) pPIL110-752, (d) pPIL110-76. Apparent *M_r*s of the proteins are indicated at the left ($\times 10^3$; K in the figure).

To localize the genes encoding these proteins, additional plasmids were constructed by deleting one or more fragments of pPIL110-75 (Fig. 3b) or by cloning discrete fragments of pPIL110-75 in pBR322 (Fig. 3c). JA221 cells harboring these plasmids did not show MRHA. As F₇₁ fimbriae are composed of subunits with apparent *M_r*s of 20000, we suppose that the 20-kDa protein expressed by pPIL110-75 represents the fimbrial subunit. The 20-kDa protein is expressed by minicells carrying pPIL110-752 (Fig. 2, lane c), but not by minicells carrying any of the other plasmids (Table 1).

Treatment of minicells harboring pPIL110-753 with 9% ethanol [17] before labeling resulted in expression of a 21 kDa protein, that might represent the precursor of the 20-kDa protein. Since this precursor was not expressed in minicells harboring pPIL110-76 or pPIL110-78 we suppose that the gene encoding this protein spans the *Hind*III site in pPIL110-753. Recent DNA sequence data have established this supposition (Van Die et al., unpublished results). These results indicate that the gene encoding the fimbrial subunit was expressed, but not properly processed (e.g., degradation might occur). A similar observation was made for the subunit of the F₇₂ fimbriae [11].

The molecular organization of the accessory genes became apparent from the analysis of the gene products encoded by deletion plasmids and subclones derived from pPIL110-75 (Fig. 2, Table 1).

The location of the different genes on pPIL110-75, deduced from the minicell experiments, is shown in Fig. 4a. The order of the *M_r* 36000 and 14000 genes in pPIL110-754 could not be deduced from the minicell experiments. We suppose, based on analogy with the F₇₂ and Pap fimbrial gene clusters [11,19], that the order of these genes is as shown in Fig. 4a. The 15-kDa, 17.5-kDa and 40-kDa proteins were not observed in all minicell experiments. Treatment of minicells harboring pPIL110-77 with 9% ethanol [17] before labeling resulted in increased expression of the 15-kDa and 40-kDa and decreased expression of the 14-kDa and 36-kDa proteins. These results suggest that the 15-kDa and 40-kDa proteins represent precursor proteins of the 14-kDa and 36-kDa proteins, re-

Table 1
Plasmid-encoded proteins of pPIL110-75 and derivatives in minicells
Next to the proteins listed all plasmids did express vector-encoded proteins.

Plasmids	Gene products expressed by minicells harbouring different plasmids [in kilodalton (kDa)]								
	75	40	36	23	20	17.5	17	15	14
pPIL110-75	+	+	+	+	+	+	+	+	+
pPIL110-752	+	-	-	+	+	+	+	-	-
pPIL110-751	+	+	+	+	-	+	+	+	+
pPIL110-77	-	-	+	-	-	-	+	-	+
pPIL110-78	-	-	-	-	-	-	-	-	-
pPIL110-76	+	-	-	-	-	-	-	-	-
pPIL110-754	-	-	+	-	-	-	-	-	+
pPIL110-755	+	-	-	+	-	-	+	-	-
pPIL110-753	-	-	-	-	-	-	-	-	-



Fig. 3. Physical maps of pPIL110-75 and derivatives. The shaded boxes represent pJB8 DNA. (a) Physical map of pPIL110-75; (b) physical map of deletion plasmids of pPIL110-75; (c) restriction fragments of pPIL110-75, cloned in pBR322; (d) physical map of pPIL110-701, derived from pPIL110-7 by cloning a *Eco*RI-*Cla*I fragment in pBR322. The black box represents pBR322 DNA.

spectively. It is not clear if the 17.5-kDa protein represents a precursor protein of the 17-kDa protein, as no increased expression was found after ethanol treatment of the minicells.

As reported recently, the MRHA phenotype is not strictly dependent on possession of fimbriae. For the Pap fimbrial gene cluster, a non-polar mutation in the fimbrial subunit did not result in loss of MRHA [18,19]. JA221 cells harboring pPIL110-751, that expresses all proteins of the F₇₁ fimbrial gene cluster except the fimbrial subunit (Fig. 2, lane b), did not show MRHA. However, JA221 cells carrying pPIL110-701 (Fig. 3d), that encompasses the genetic information present in pPIL110-751, did show MRHA but did not agglutinate in antiserum raised against F₇₁ fimbriae, indicating that no fimbriae were formed. No fimbriae were observed either on electron microscopic examination. In pPIL110-701 the accessory genes are located directly downstream of the anti-promoter of the tetracycline resistance gene of pBR322 [20]. Apparently, expression of MRHA is dependent on a promoter in front of the *M_r* 75 000 gene that is stronger than the promoter present in pPIL110-751. We conclude that also for the F₇₁ gene cluster, as was shown before for the Pap fimbrial gene cluster, the MRHA phenotype is not strictly dependent on expression of fimbriae.

4.4. Comparison of the *F7₁* and *F7₂* gene clusters

Some differences, however, are observed between the gene clusters. The F7₁ gene cluster causes a clear expression of proteins with a M_r of 14 000 (D' gene product) and 17 000 (F' gene product). In the Pap-gene cluster expression of proteins with a similar M_r (products of papE and papF) was observed from the corresponding DNA fragment [17]. The corresponding region of the F7₂ gene cluster, however, does not cause detectable expression of similar gene products in minicells, although a functional complementation of the D-gene of F7₂ is accomplished by the F7₁ D' gene. We suppose that expression of the F7₂ gene cluster is much lower compared to the F7₁ gene cluster.

Table 2

Complementation of transposon insertion mutations in pPIL110-35 (F₇₂) by F₇₁ derivatives

JA221 carrying F ₇₁ plasmids	MRHA phenotype of JA221 carrying Tn 5 insertion plasmids in genes A-E of pPIL110-35 (F ₇₂)				
	Gene A [6]	Gene B [11]	Gene C [16]	Gene D [21]	Gene E [25]
pPIL110-752	+	+	+	—	—
pPIL110-754	—	—	—	+	+

Lindberg et al. [15] suggest that the DNA region encompassing papE, papF and papG is involved in expression of the adhesion factor. The low MRHA activity of JA221 cells carrying the F₇₂ clone pPIL110-35 (MRHA titer 1/32) compared to JA221 cells carrying the F₇₁ clone pPIL110-75 (MRHA titer 1/128) might be due to a lower expression of this DNA region in the F₇₂ gene cluster.

It is striking that AD110 harbors two functionally related gene clusters coding for the biosynthesis of P-fimbriae. Such a situation might also exist in *E. coli* strain KS71, which has a different OK serotype but appears to produce similar fimbriae as AD110 [21,22]. Further studies should elucidate the origin of the two gene clusters and answer the question whether products of the two gene clusters are exchanged resulting for instance in 'mixed' fimbriae or whether expression is regulated by phase variation, as is shown for the C and P fimbriae of strain KS71 [23].

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