Molecular cloning of a determinant coding for fimbrial antigen F165₁, a Prs-like fimbrial antigen from porcine septicaemic *Escherichia coli*

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The genetic determinant coding for F165₁ fimbriae was cloned from the chromosome of the porcine *Escherichia coli* wild-type strain 4787 (O115:K⁻:H51:F165). The fimbrial determinant was further subcloned into the *Bam*HI site of pACYC184 and a restriction map was established. On Southern hybridization, identity between the chromosomally encoded *prs*-like determinant of strain 4787 and its cloned counterparts was demonstrated. The cloned F165₁ fimbriae and those of the wild-type strain possessed a major protein subunit of molecular mass 18.5 kDa. Strains expressing F165₁ fimbriae were detected using an F165-specific polyclonal antiserum and caused mannose-resistant haemagglutination and agglutination of Forssman latex beads. Antiserum against the cloned F165₁ fimbriae recognized a 18.5 kDa band in the parent strain 4787.

Introduction

Extra-intestinal *Escherichia coli* are a frequent cause of urinary tract infection, sepsis and newborn meningitis (NBM) (Ørskov & Ørskov, 1985; Svanborg-Edén & De Man, 1987). Extra-intestinal *E. coli* strains produce adhesins which mediate attachment to eukaryotic cells and which can be distinguished by their receptor specificity (Hacker, 1989; Tennent *et al.*, 1990). Common F1A (type 1) fimbrial adhesins recognize α -mannose in glycoproteins (Ørskov & Ørskov, 1983), whereas mannose-resistant adhesins bind to eukaryotic receptor substances other than mannose. P fimbriae, which represent the main group of mannose-resistant fimbriae associated with urinary tract infections, possess G-adhesins, which recognize the globo-series of glycolipids present on human erythrocytes possessing the P blood group antigen (Källenius *et al.*, 1980; Väisänen *et al.*, 1984; Strömberg *et al.*, 1991). The *prs* operon, which is very similar to the operon coding for P fimbriae, encodes PrsG, which preferentially binds to the Forssman antigen, a major constituent on sheep erythrocytes (Lund *et al.*, 1988; Lindstedt *et al.*, 1989; Karr *et al.*, 1989).

Another important group of mannose-resistant adhesins, the S fimbrial adhesins, recognize an α -sialyl-(2 $\rightarrow$ 3) β -Gal-containing receptor structure and are commonly found on *E. coli* strains causing sepsis or newborn meningitis (Korhonen *et al.*, 1984; Hacker *et al.*, 1985). In addition to fimbrial adhesins, other adhesive proteins of extra-intestinal *E. coli*, which do not exhibit fimbrial structures, have been observed (Labigne-Roussel *et al.*, 1984; Rhen *et al.*, 1986).

A mannose-resistant fimbrial antigen, F165, on O115 strains causing septicaemia in young pigs has been identified (Fairbrother *et al.*, 1986). Fimbrial antigen F165 is found on *E. coli* strains isolated from piglets and calves with septicaemia and/or diarrhoea (Fairbrother *et al.*, 1988*b*). F165 fimbriae are long, rigid and have a diameter of about 5 to 8 nm (Fairbrother *et al.*, 1988*a*). The purified fimbrial complex consists of two separate protein subunits, with apparent molecular masses of 19000 and 17500 Da, which we have subsequently designated F165₁ and F165₂, respectively (Fairbrother *et al.*, 1988*a*). It has been demonstrated that *pap* sequences or *pap*-related sequences are predominant in F165-positive isolates from animals and may be found in association with *afa* or *sfa* or related sequences (Harel *et al.*, 1991*a*). F165-positive isolates may express multiple adhesins, two of which recognize the GalNAc(α 1 $\rightarrow$ 3)- β GalNAc moiety of the Forssman antigen and the Gal(α 1 $\rightarrow$ 4) β Gal moiety of the P blood group antigen

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Abbreviation: MRHA, mannose-resistant haemagglutination.

respectively (J. Fairbrother and others, unpublished). Expression of the Forssman-recognizing adhesin appears to be associated with pathogenicity of F165-positive strains in intraperitoneally inoculated adult mice and in orally inoculated newborn pigs (Fairbrother *et al.*, 1989).

This study reports the cloning of a genomic DNA fragment required for the expression of F165₁ fimbriae, one of the fimbrial components of F165 complex, which recognizes the GalNAc(α 1 $\rightarrow$ 3)GalNAc moiety of the Forssman antigen, from a porcine septicaemic F165-positive *E. coli* O115 strain.

Methods

Bacterial strains and plasmids. *E. coli* 4787 is a wild-type strain (O115:K⁻:H51:F165) isolated from the intestinal contents of a diarrhoeic piglet. Strain 4787 was shown to contain P- and S-related nucleotide sequences (Harel *et al.*, 1991a). *E. coli* HB101 *hsdR hsdM recA13 supE44 lacZ4 leuB6 proA thi-1 Str^r* was used as the host in transfection and transformation experiments. Vector plasmids pMMB33 (Frey *et al.*, 1983) and pACYC184 (Chang & Cohen, 1978) were used for cloning experiments. Plasmid pPIL1006 carrying a *papC* gene internal fragment (Archambaud *et al.*, 1988) was used in colony hybridization experiments.

Bacteria were cultivated in L-broth or on L-agar plates containing 1.5% (w/v) agar (Miller, 1972). Selective pressure against loss of plasmids was imposed by adding 50 µg kanamycin ml⁻¹ or 20 µg chloramphenicol ml⁻¹ to the medium. Strains were grown on minimal medium for maximal expression of F165 (Fairbrother *et al.*, 1988a).

Cosmid cloning. Genomic DNA from *E. coli* 4787 was partially cleaved with the restriction enzyme *Sau*3A and ligated into the *Bam*HI site of cosmid vector pMMB33 DNA digested with *Bam*HI, *Hpa*I and *Sma*I (Frey *et al.*, 1983). Cosmids were packaged into phage λ particles and used to infect *E. coli* HB101 (Hull *et al.*, 1981). Following selection, subcloning was done into the *Bam*HI site of pACYC184.

Isolation, transformation and analysis of plasmid DNA. The method of Birnboim & Doly (1979) was used for isolation of plasmid DNA from *E. coli* HB101 host strains and wild-type F165-positive *E. coli* 4787. Plasmid DNA was extracted and purified by ultracentrifugation in caesium chloride/ethidium bromide gradients (Maniatis *et al.*, 1982). Competent cells prepared by the calcium chloride/rubidium chloride method were used for plasmid transformation to *E. coli* (Kushner, 1978). Plasmids were digested with appropriate restriction endonucleases under conditions specified by the manufacturer (Pharmacia-LKB). Electrophoretic analysis of plasmid DNA was performed using 0.7% agarose gels and Tris/borate buffer. Restriction fragments were purified by extraction from low melting agarose after electrophoresis (Lortie *et al.*, 1991).

Southern hybridization and autoradiography. Transfer of DNA fragments from agarose gels to nitrocellulose filters, washing and autoradiography were done as described by Southern (1975). Stringent conditions were used for the washing procedure. Colony hybridizations were carried out as described by Harel *et al.* (1990). Appropriate DNA fragments were cut out of the gel, purified, and radiolabelled with [α -³²P]dCTP by using a random oligolabelling kit (Pharmacia-LKB) according to the manufacturer's instructions (Harel *et al.*, 1991b).

Haemagglutination. Mannose-resistant haemagglutination (MRHA) was assessed essentially as described by Fairbrother *et al.* (1988a) using human OP₁ and A₁P₁ erythrocytes, and porcine and ovine erythro-

cytes. Erythrocytes were diluted to 3% (v/v) suspensions in phosphate-buffered saline (PBS; 0.85% NaCl) containing 2.5% (w/v) methyl α -mannoside. Erythrocytes and bacterial suspensions (20 µl of each) were mixed on a glass slide. The slide was tilted and agglutination was observed visually.

Latex bead agglutination. Latex beads (20 µl) coated with Gal(α 1 $\rightarrow$ 4)Gal β (P1 BSA; Ag latex, Chembiomed, Canada) or GalNAc(α 1 $\rightarrow$ 3)GalNAc β (Forssman disaccharide latex, Chembiomed) were mixed on a slide with an equal volume of bacterial suspension at 4 °C. After tilting for 2 min, agglutination was read (Labigne-Roussel *et al.*, 1984). All reagents and suspensions were kept at 4 °C.

Antiserum production. Polyclonal antibodies were generated against F165 complex purified by FPLC from *E. coli* 4787 by injection of 10 µg aliquots of purified proteins into rabbits as described previously (Fairbrother *et al.*, 1986). To enhance the specificity of immunodetection of fimbrial proteins, the antibodies were absorbed against whole cells of strain 4787 grown at 18 °C. F165₁ fimbrial antiserum was prepared by immunization of rabbits with a crude fimbrial preparation from HB101(pIIIA9) as described previously (Contrepois *et al.*, 1989). This antiserum was absorbed repeatedly with strain HB101 to remove antibodies not specific for the fimbrial antigen.

Immunocolony. Production of F165 by the clones was determined using an immunocolony technique on bacteria grown on MD-1 agar. Bacteria were spotted on nitrocellulose filters. After drying, the filters were saturated with 5% (w/v) skim milk and incubated for 90 min at 25 °C with F165 complex antiserum (dilution 1/1000) in Tris/buffered saline (TBS) (0.2 M-Tris/HCl, 0.5 M-NaCl, pH 7.5) containing 0.05% (v/v) Tween 20 (TTBS) plus 2.5% skim milk. Filters were then incubated for 1 h at 25 °C in 1/5000 diluted goat anti-rabbit immunoglobulin G. After a short wash in TTBS, the filters were incubated in 1/3000 avidine peroxidase. Finally, the filters were washed twice in TTBS and twice in TBS and treated with hydrogen peroxide substrate and 4-chloronaphthol chromogen.

Immuno-electron microscopy. A drop of a washed bacterial suspension was placed on Formvar-coated grids for 1 min. The grids were then placed sequentially on drops of PBS containing 1% (w/v) ovalbumin for 5 min and a suitable dilution of rabbit F165 complex antiserum for 30 min. Grids were washed and placed on drops containing gold colloidal particles (10 nm) conjugated to protein A (Sigma) for 30 min. After a final washing step, the grids were stained with 1% (w/v) phosphotungstic acid (pH 7.0) and examined with an electron microscope (Phillips 201) at an accelerating voltage of 60 kV (Broes *et al.*, 1988).

Isolation of fimbriae, PAGE and immunoblotting. Crude fimbrial extracts were prepared from bacteria grown on MD-1 plates as described previously (Fairbrother *et al.*, 1988a). After treatment of the fimbrial samples by boiling for 5 min in 10 mM-Tris/HCl (pH 7.8) containing 4% (w/v) SDS, 0.01 M-mercaptoethanol, 0.2 M-glycerol and 0.002% bromophenol blue, the samples were run on slab gels as described previously (Fairbrother *et al.*, 1988a). Western blotting (immunoblotting) was carried out by the method of Towbin *et al.* (1979). Crude fimbrial extracts from wild-type *E. coli* 4787, *E. coli* HB101(pIIIA9) and HB101(pCJ7) were electrophoresed on SDS-polyacrylamide gels. Following transfer to nitrocellulose filters, the electrophoresed preparations were reacted with F165 complex and F165₁ fimbrial antisera to identify the fimbrial bands.

Amino acid composition analysis. Protein was electroblotted on Immobilon transfer membrane (Millipore) following the method of Legendre & Matsudaira (1988). Amino acid sequencing of specific protein bands was done on an Applied Biosystems model 470A gas phase Sequanator running a standard operating program. Phenylthio-

hydantoin derivatives were separated using an on-line PTH analyser (Applied Biosystems, model 900).

Results

Molecular cloning of the F165₁ fimbriae determinant

Genomic DNA was isolated from strain 4787 possessing the F165 fimbrial complex. After cleavage with *Sau*3A and a packaging procedure, 600 *E. coli* K12 colonies were screened for the components of the F165 complex by the immunocolony detection technique. One of the recombinant clones harbouring a cosmid, designated pIIIA9, that was found to react with F165 complex antiserum, was also *pap*-positive by colony hybridization. The expression by this clone of fimbriae reacting with F165 complex antiserum was confirmed by the isolation of fimbriae and by subsequent SDS-PAGE and immunoblotting with F165 antiserum. The MRHA haemagglutination and receptor binding properties (Table 1) indicated the presence of *prs*-like, rather than *pap*, coding genes.

The pIIIA9 DNA was further cleaved with the restriction enzyme *Bam*HI, and suitable fragments were ligated into the *Bam*HI site of pACYC184. A recombinant plasmid, pCJ7, had lost tetracycline resistance, still expressed resistance to chloramphenicol and conferred on strain HB101 specific agglutination of Forssman latex beads and of ovine, porcine and human agglutination group A₁P₁ erythrocytes, and expression of fimbriae. Because of their haemagglutination and receptor binding properties, the clones derived from the *pap*-positive cosmid were described as *prs*-like clones. Electron microscopy showed that the surface of the bacteria was fimbriated. The structures could be identified by immunogold labelling experiments as F165 fimbriae using F165 complex antiserum (Fig. 1).

Fig. 1. Transmission electron micrograph of *E. coli* HB101(pCJ7) labelled using the immunogold technique. Cells were incubated with antiserum raised against F165 fimbriae and then with protein A-gold (10 nm). Bar, 1 µm.

Restriction map of pCJ7

The recombinant plasmid pCJ7 consisted of the vector pACYC184 and a chromosomal insert of 10 kb. The physical map of pCJ7 was further determined by digestion with different restriction enzymes (Fig. 2). The

Table 1. Characteristics of wild-type *E. coli* strain 4787 and subclones carrying various plasmids

Strain/ plasmid	Geno- type	Mannose-resistant haemagglutination			Agglutination of latex beads	
		Human erythrocytes		Ovine and porcine erythrocytes		
		A ₁ P ₁	OP ₁		Gal1→4Gal	GalNAc1→4GalNAc
4787d	<i>pap sfa</i>	+++	—	+++	(+)	+++
pIIIA9	<i>pap</i>	+	—	+	—	+++
pCJ7	<i>pap</i>	+++	—	+++	—	+++
HB101	—	—	—	—	—	—

Fig. 2. Alignment of *F165*₁, *pap* and *prs* gene clusters. Restriction sites which map at identical positions in the clusters are indicated with arrows on pPAP5 and pPAP601 (Lund *et al.*, 1988).

results indicated that the *prs*-like gene cluster is physically related to both the *pap* and *prs* gene clusters (Lund *et al.*, 1988; Lindstedt *et al.*, 1989; Karr *et al.*, 1989). A *Bgl*II site, which is present in *papG* but absent in *prsG*, was not found in pCJ7. In contrast to the *prs* operon, some restriction endonuclease sites, such as *Hpa*I and *Eco*RI sites, are found in the 3' region of the *F165* operon of pCJ7 (Fig. 2).

*Pst*I-cleaved chromosomal DNA of strain 4787 and pCJ7 plasmid DNA were hybridized against an α -³²P-labelled probe for the F165₁ determinant from pCJ7, consisting of the 10 kb *Bam*HI fragment. As judged from the restriction maps of other cloned P determinants which share some homology, this fragment carries most of the internal F165₁ coding region. Identical *Pst*I fragments of 1.8, 1.2, 0.5 and 0.3 kb were obtained from the genomic and recombinant plasmid DNA (Fig. 3).

The location of the genes expressing MRHA in the wild-type parent strain 4787 was determined. Strain 4787 was the host of at least five plasmids of different molecular masses. The plasmids were purified from strain 4787, digested with *Pst*I, and hybridized with the *Bam*HI probe for pCJ7 (Fig. 3). None of the plasmid-generated fragments exhibited homologous sequences. These results showed that the genes associated with MRHA expression in strain 4787 were not plasmid-encoded and that four *Pst*I internal fragments from strain 4787 chromosomal DNA were present. Moreover, the *F165*₁ operon was present in a single copy in wild-type strain 4787, as demonstrated by a unique 19 kb *Bam*HI chromosomal band hybridizing with the pCJ7 *Bam*HI probe and containing an internal *Eco*RI site (Fig. 3).

Characterization of the F165₁ fimbriae

After purification of the fimbriae and dissociation with SDS, an 18.5 kDa protein band was obtained in PAGE from both the wild-type strain 4787 and the *E. coli* K12 clones harbouring pIIIA9 or pCJ7. A second band of

Fig. 3. Hybridization patterns of *Pst*I-cleaved plasmid DNA from *E. coli* strain 4787 (lane 1), HB101(pCJ7) (lane 2), chromosomal DNA from *E. coli* 4787 (lane 3), *Bam*HI-cleaved (lane 4), and *Eco*RI-cleaved (lane 5) chromosomal DNA from *E. coli* 4787. The hybridization probe was the α -³²P-labelled 10 kb *Bam*HI fragment of pCJ7.

17 kDa was visible in preparations of *E. coli* 4787. Wild-type strain 4787 and strains carrying the cloned *prs*-like determinant reacted strongly with the F165 complex antiserum in the immunocolony assay, whereas strain HB101 showed no reaction. Immunoblotting using F165 complex antiserum revealed two bands of 18.5 kDa and 17 kDa in the parent strain 4787, whereas in subclone HB101(pCJ7) only one band of 18.5 kDa, F165₁, was revealed (Fig. 4). Moreover, antiserum directed against F165₁ crude extract from HB101(pIIIA9) recognized only one band of 18.5 kDa in the parent strain 4787, and one band in HB101(pIIIA9) and HB101(pCJ7) (Fig. 4). The second band of 17 kDa, visible in preparations of *E. coli* 4787 reacted with F165 complex antiserum, is the

Fig. 4. Western blot of SDS-PAGE of fimbrial subunits from different *E. coli* strains. In (a) the nitrocellulose was reacted with rabbit F165₁ (Prs-like) antiserum to clone pIIIA9; in (b) the nitrocellulose was reacted with rabbit F165 antiserum to strain 4787. Lanes 1, strain 4787; lanes 2, HB101(pCJ7). The position of the 18.5 kDa band is indicated (◄).

Table 2. Amino-terminal amino acid sequences of F165₁, PapA, F7₂, F11 and F7₁ fimbriae

a, Båga *et al.* (1984); b, Van Die & Bergmans (1984); c, Van Die *et al.* (1987); d, Rhen *et al.* (1985).

	5	10	14
F165 ₁	APTTP	QGQGI	VTFN
PapA ^a	APTTP	QGQGI	VTFN
F7 ₂ ^b	APTIP	QGQGI	VTFN
F11 ^c	APTIP	QGQGI	VTFN
F7 ₁ ^d	AASIP	QGQGE	VSFK

major protein subunit of fimbrial component F165₂ (Harel *et al.*, 1991a).

Amino-terminal amino acid sequencing revealed that from residue 1 to 14, the sequence of the 18.5 kDa F165₁ fimbrial major subunit closely resembled that of PapA (Båga *et al.*, 1984), F11 (Van Die *et al.*, 1987) and F7₂ (Van Die & Bergmans, 1984) fimbriae and, to a lesser extent, that of F7₁ fimbriae (Rhen *et al.*, 1985) (Table 2). The amino-terminal sequence differed from those of Pap, F11 and F7₂ at two sites with a substitution of isoleucine for threonine at position 4 and a substitution of leucine for isoleucine at position 10.

Discussion

In this report, the molecular cloning of the gene encoding the F165₁ fimbrial component of the F165 complex from an *E. coli* strain that can cause septicaemia in pigs is

described. After a cosmid cloning procedure, a 10 kb piece of DNA was inserted into the *Bam*HI site of pCAYC184. This chromosomal DNA insert conferred MRHA specific for Forssman adhesins (F adhesins) and the formation of fimbriae related to P fimbriae. On the basis of restriction sites, some similarities were revealed between the cloned Prs-like determinant and *pap* and *prs* determinants coding for P and Prs fimbriae (Hull *et al.*, 1981; Lund *et al.*, 1989; Karr *et al.*, 1989). Thus, it appears that the 10 kb DNA fragment which resulted from *Bam*HI cleavage of pIIIA9 carried most of the F165₁ coding region. When this fragment was used as a radioactively labelled probe, it revealed identity with *Pst*I fragments of the chromosomal DNA and the cloned F165₁ fimbrial plasmid DNA. It is interesting to note that, in contrast to the uropathogenic *E. coli* strain J96 which contains the *pap* and *prs* operons, strain 4787 contains only one copy of the *prs*-related sequences operon.

The molecular mass of the F165₁ major fimbrial protein subunit was found to be 18.5 kDa, which is in good agreement with the value of 19 kDa for one of the F165 complex components described by Fairbrother *et al.* (1988a). No differences in size could be seen between F165₁ fimbriae isolated from the wild-type strain and from the K12 clones harbouring the cloned F165₁ DNA. It has been suggested that F165 complex is composed of two separate protein subunits (Fairbrother *et al.*, 1988a). This would be consistent with the finding that *E. coli* strains associated with extra-intestinal infections, such as urinary disease, often produce more than one fimbrial antigen (Hacker, 1989; Svanborg-Edén & De Man, 1987). As demonstrated by immunoblotting, strain 4787 expresses at least two fimbrial components: the 18.5 kDa F165₁ fimbrial major protein subunit, and the 17 kDa F165₂ major fimbrial protein subunit related to S; the latter seems to be the predominant component in *in vitro* conditions and has recently been purified (D. Dubreuil and others, unpublished). Amino-terminal amino acid sequencing revealed that from residue 1 to 14, the sequence of the major 18.5 kDa protein subunit of fimbriae F165₁ closely resembled that of PapA (Båga *et al.*, 1984), F11 (Van Die *et al.*, 1987) and F7₂ (Van Die & Bergmans, 1984) fimbriae.

Thus, the paper describes fimbrial antigen F165₁, which genetically belongs to the P fimbrial class, but possesses an adhesin having a different structure and receptor specificity from Gal(α1→4)Gal-binding G-adhesins hitherto associated with P fimbriae. In fact, F165₁-positive clones have been characterized and show adhesion to the terminal GalNAc(α1→3)GalNAc of Forssman antigen. Moreover, the recombinant clones recognize human erythrocytes of blood group A₁P₁, but not of group O₁P₁. This indicates that the clones produce

a G-adhesin or Prs recognizing an epitope shared by the globo-A and Forssman structures, e.g. the terminal GalNAc(α 1 $\rightarrow$ 3) but not the P antigens. The Forssman antigen is a major constituent of sheep erythrocytes and is found in many species of animals. Thus, this Prs adhesin may have an important function in the infectious process of septicemic strain 4787.

Similarly, some P-antigen-recognizing strains, termed ONAP (for O-negative, A-positive) have been shown to recognize an epitope shared by globo-A and Forssman structures, e.g. GalNAc(α 1 $\rightarrow$ 3) bound to Gal(α 1 $\rightarrow$ 4)-Gal β -containing glycolipids (Lindstedt *et al.*, 1989, 1991). Orino & Naiki (1990) described the existence of two different P-fimbrial variant adhesin subunits that haemagglutinate sheep erythrocytes in the presence of D-mannose. One subunit recognizes Forssman glycosphingolipid but not P- and P^k-antigenic glycosphingolipids and the other recognizes a common carbohydrate epitope shared by Forssman glycosphingolipid, and by P- and P^k-antigenic glycosphingolipids.

The operon responsible for the fimbrial adhesin termed Prs (for P-related sequence) or Pap-2-recognizing Forssman antigen has been cloned from uropathogenic *E. coli* J96. The clones pPAP601 (Lund *et al.*, 1988) and pJK102 (Karr *et al.*, 1989), both derived from uropathogenic *E. coli* isolate J96, were constructed differently and independently. The restriction maps of these clones suggest that the gene clusters are identical based on restriction site mapping. A structural difference between the *pap* and *prs* gene clusters was located within the *papG* region. *prsG* encodes an adhesin-recognizing receptor on goat and sheep erythrocytes containing large quantities of Forssman glycolipids (Strömberg *et al.*, 1991). The *prs*-like clone pCJ7, derived from animal *E. coli* strain 4787, has a restriction map similar to the *prs* and *pap-2* clones, but with some differences at the 3' end. It is not known as yet whether the adhesin expressed by pCJ7 differs structurally from PrsG. Nevertheless, the adhesin from pCJ7 must be sufficiently related to PrsG in order to recognize a similar class of erythrocytes but to differ from PrsG adhesin, as the latter does not agglutinate porcine erythrocytes (Strömberg *et al.*, 1991).

F165-positive *E. coli* isolates have many of the virulence determinants found in extra-intestinal *E. coli*, such as aerobactin production and resistance to the bactericidal effects of serum and possess *pap* sequence alone or in association with *afa* or *sfa* (Fairbrother *et al.*, 1988a; Harel *et al.*, 1991a). However, the role of P and Prs adhesins in infections caused by F165-positive isolates is not known. The important role of P adhesins in the physiopathology of *E. coli* pyelonephritis in humans has been reported in several studies. Few reports have described the presence of *pap*-related operons in porcine or bovine *E. coli* isolates (Harel *et al.*, 1991a; Ott *et al.*,

1991). The strains with specificity for globo-A and the Forssman glycolipid were first isolated from the urinary tract of dogs (Garcia *et al.*, 1982; Senior *et al.*, 1988). P-positive *E. coli* have also been found in dogs with urinary tract infection (Low *et al.*, 1988; Strömberg *et al.*, 1990) and in avian septicemia (Achtman *et al.*, 1986). Gastra and others (reported in Tennent *et al.*, 1990) have demonstrated that *E. coli* isolated from dogs with urinary tract infections frequently express fimbriae that react with monoclonal antibodies directed against the fimbrial subunits of P fimbriae F12 and F13. Low *et al.* (1988) have found dog urinary tract infection *E. coli* isolates that contain genes homologous to *pap* but express a neuraminidase-sensitive binding phenotype. A high prevalence of PrsG-like specificities has been observed among wild-type uropathogenic (dog) *E. coli* (Strömberg *et al.*, 1990).

Thus, a genomic DNA fragment coding for F165₁, one of the fimbrial components of F165 complex, with a structural subunit of 18.5 kDa has been cloned. The introduction of this Prs-like determinant into various wild-type hosts offers possibilities for the construction of novel live vaccines in animals, as already suggested for the *E. coli* K88 adhesin (Bakker *et al.*, 1990).

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