Cloning of determinants encoding F165₂ fimbriae from porcine septicaemic *Escherichia coli* confirms their identity as F1C fimbriae

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Cloning of the *f165*₂ operon that encodes F1C-like fimbriae in *Escherichia coli* indicates that this operon is a member of the S/Foc family. The genetic determinant coding for the F165₂ fimbriae was cloned from the chromosome of the porcine *E. coli* wild-type strain 4787 (O115:K⁻:H51:F165). The cloned F165₂ and the wild-type operon expressed a major fimbrial protein subunit of molecular mass 17·2 kDa that was detected by anti-F165 and anti-F1C polyclonal sera. The sequences of the *f165*_{2A} and *f165*_{2FGH} genes are reported. Major subunit gene *f165*_{2A} encodes a mature protein of 156 amino acids. Minor subunit genes *F*, *G* and *H* encode mature proteins of 148, 145 and 276 amino acids, respectively. The amino acid sequences of the four proteins share similarities with those of the known S and F1C fimbrial antigens that are produced by extraintestinal *E. coli* which is associated with sepsis, urinary tract infections and newborn meningitis. The F165_{2A} protein was identical to the major subunit protein of F1C, with a difference only at the first position. It was also similar, to a lesser extent, to the major subunit proteins of SfaI and SfaII fimbriae. F165_{2F} was identical to FocF and SfaG/SfaII G. F165_{2G} was more closely related to FocG than to SfaI/SfaII S, and F165_{2H} was more closely related to FocH than to SfaI/SfaII H.

Keywords: extraintestinal *Escherichia coli*, *f165*₂ fimbrial operon, fimbrial subunit sequences

INTRODUCTION

Fimbrial antigen F165, which exhibits mannose-resistant haemagglutination (MRHA) of sheep, pig and human type A erythrocytes is produced by *Escherichia coli* O115 isolates causing septicaemia in young pigs (Fairbrother *et al.*, 1993). In septicaemic *E. coli* strain 4787 (O115:K⁻:H51), F165 fimbrial antigen complex consists of two fimbrial components, F165₁ and F165₂, of approximately 18·5 and 17·2 kDa respectively (Fairbrother *et al.*, 1986, 1988; Dubreuil & Fairbrother, 1992). It was suggested that F165 could contribute to the pathogenicity of these isolates (Fairbrother *et al.*, 1989; 1993). The

cloned *f165*₁ operon from strain 4787 expresses a major fimbrial subunit of 18·5 kDa (Harel *et al.*, 1992a). The genetic determinant of fimbrial antigen F165₁ belongs to the P fimbrial class. The *f165*₁ operon encodes an adhesin whose MRHA and receptor binding properties are similar to those of a Prs-like adhesin recognizing the Forssman antigen of sheep erythrocytes (Harel *et al.*, 1992a; Maiti *et al.*, 1993). *TnpA* mutagenesis studies have demonstrated that the presence of the F165₁ fimbrial system is required for complete pathogenicity of at least one F165-positive strain in germ-free pigs (Harel *et al.*, 1992b; Ngeleka *et al.*, 1993). A second fimbrial component of 17·2 kDa, from *E. coli* 4787 was purified and was named F165₂ (Dubreuil & Fairbrother, 1992). For the first 33 amino acid residues, the F165₂ sequence was identical to that of F1B and very similar to that of F1C fimbriae associated with *E. coli* isolates from urinary tract infections (UTI) in humans (Klemm *et al.*, 1982; van Die *et al.*, 1984; Dubreuil & Fairbrother, 1992).

Abbreviations: MRHA, mannose-resistant haemagglutination; UTI, urinary tract infection.

The GenBank accession numbers for the sequences reported in this paper are U09857 and U09804 for *f165*_{2A} and *f165*_{2FGH}, respectively.

Fimbrial adhesins produced by extraintestinal *E. coli*, associated with diseases such as UTI sepsis, and newborn meningitis, are important surface antigens and mediate attachment to eukaryotic epithelial cells (Ørskov & Ørskov, 1983; Hacker, 1990; Tennent *et al.*, 1990; Johnson, 1991). Commonly found fimbriae in human extraintestinal *E. coli* are P, S, M and X (Korhonen *et al.*, 1985; Labigne-Roussel & Falkow, 1988). S fimbrial adhesin (Sfa), which is associated to a larger extent with strains causing newborn meningitis and less often with uropathogenic strains, interacts with α -sialyl-(2-3)- β -D-Gal-containing receptors and agglutinates bovine erythrocytes (Hacker *et al.*, 1985). Another type of fimbria, F1C, is associated with UTI (Riegman *et al.*, 1990). F1C and S fimbriae belong to the same family of fimbrial adhesins, related genetically, immunologically and functionally (Marre *et al.*, 1990; Ott *et al.*, 1988; Riegman *et al.*, 1990). Although F1C fimbriae lack any haemagglutinating activity, they are able to bind to uroepithelial cells. In both *sfa* and *foc* gene clusters, which encode the S and F1C fimbriae respectively, the distal parts encode minor fimbrial subunits (van Die *et al.*, 1991; Hacker, 1985; Schmoll *et al.*, 1989; Riegman *et al.*, 1990). For the S fimbriae, the 15 kDa protein SfaS is the adhesin that binds to carbohydrate chains terminating with α -sialyl-(2-3)- β -D-Gal-R (Parkkinen *et al.*, 1986; Moch *et al.*, 1987; Morschhäuser *et al.*, 1990), whereas the adhesin of F1C fimbriae is not known. F1C fimbriae as well as S-fimbriated adhesins were shown to mediate binding to renal tubular cell lines (Virkola *et al.*, 1988; Marre *et al.*, 1990). Sfr (for S/F1C related) fimbriae show serological cross-reactivity with S fimbriae and F1C, but fail to exhibit S-specific binding properties (Pawelzik *et al.*, 1988).

The study reports the cloning of a genomic DNA fragment required for the expression of F165₂ fimbriae, one of the fimbrial components of F165 complex, from a septicaemic porcine F165-positive *E. coli* O115 strain. We also report the nucleotide sequences encoding the major A subunit and minor F, G, and H subunits of F165₂, and the relationship of this fimbrial antigen, which is expressed by *E. coli* causing septicaemia in pigs, with members of the S/F1C family, which is expressed by *E. coli* causing UTI and newborn meningitis in humans.

METHODS

Bacterial strains and plasmids. *E. coli* 4787 is a wild-type strain (O115:V165: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.*, 1991; Maiti *et al.*, 1993). *E. coli* HB101 (*hsdR hsdM recA13 supE44 lacZ4 leuB6 proA thi-1 Sm^r*) was used as the host in transfection and transformation experiments. *E. coli* DS910 (*minB thi rpsL recA*), kindly provided by G. Szatmari (University of Montreal), was used for minicell experiments. Vector plasmids pMMB33 (Frey *et al.*, 1983), pACYC184 (Chang & Cohen, 1978) and pCR (Invitrogen) were used for cloning experiments. Plasmid pPIL110-51, derived from strain D1990 containing the genetic determinant expressing F1C fimbriae, was kindly provided by I. Ørskov (van Die *et al.*, 1984). Plasmid pPIL1007, carrying an *sfaDE* gene internal fragment (Archambaud *et al.*, 1988),

kindly provided by A. Labigne (Services des Entérobactéries, INSERM, Institut Pasteur, Paris, France), was used in colony hybridization experiments. *E. coli* Inv- α -F' (*endA1 hsdR17 (r_K⁻, m_K⁺) supE44 thi recA1 gyrA relA ϕ 80 lacZ Δ M15 Δ (lacZYA-argF) *deoR*⁺) was used as host, and plasmid pCR was used as the vector for subcloning. Bacteria were cultivated in L-broth or on L-agar plates containing 1.5% (w/v) agar (Sambrook *et al.*, 1989). Selective pressure against loss of plasmids was imposed by adding 50 μ g kanamycin ml⁻¹ or 20 μ g chloramphenicol ml⁻¹ to the medium. Strains were grown on minimal Davis (MD-1) medium for the maximum expression of F165 (Fairbrother *et al.*, 1988). Plasmid DNA was isolated by CsCl/ethidium bromide density-gradient centrifugation (Sambrook *et al.*, 1989).*

Recombinant DNA techniques. Restriction endonucleases were obtained from Pharmacia and Baie d'Urfé, and were used according to the manufacturers' specifications. DNA restriction fragments were separated on low-melting agarose gels (1%, w/v) and the DNA fragments were eluted from the agarose by heating to 65 °C and extracting with phenol (Sambrook *et al.*, 1989). Ligation of DNA fragments was performed with T4 DNA ligase and transformation into the *E. coli* host was done by electroporation (Bio-Rad) as described previously (Harel *et al.*, 1992a).

Nucleotide sequencing. The nucleotide sequence of the *f165₂A* and *f165₂FGH* genes of cloned inserts in pCR was determined on double-stranded DNA by the dideoxy chain-termination method using the T7 deaza sequencing kit (Pharmacia). Primers flanking the cloned inserts and internal oligonucleotide primers were used to sequence the inserts. Oligonucleotides were synthesized on a Gene Assembler Plus (Pharmacia). The programs used for compiling the nucleotide and amino acid sequence data were from GCG and Multiple Alignment (Devereux *et al.*, 1984; Corpet, 1988; Kyte & Doolittle, 1982).

Production of antisera. Polyclonal antibodies were generated against purified F165 fimbriae from strain 4787, and purified F1C fimbriae from strain HB101 containing pPIL110-51 (van Die *et al.*, 1984), by injecting 10 μ g aliquots of purified proteins into rabbits as described by Harel *et al.* (1992a).

Cosmid cloning. Genomic DNA from *E. coli* 4787 was partially cleaved with *Sau3A* 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.

Southern hybridization and autoradiography. Transfer of DNA fragments from agarose gels to nitrocellulose filters, washing and autoradiography were carried out as described by Southern (1975) and Harel *et al.* (1992a). Stringent conditions were used for the washing procedure. Colony hybridizations were carried out as described by Harel *et al.* (1991).

Haemagglutination. MRHA was carried out essentially as described by Fairbrother *et al.* (1988) using human A₁P₁, porcine, bovine and ovine erythrocytes.

Immunoelectron 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 with 1% (w/v) ovalbumin for 5 min and a suitable dilution of rabbit anti-F1C serum for 30 min. Grids were washed and placed on drops of anti-rabbit immunoglobulin with 10 nm gold particles (Sigma) for 30 min. After a final washing step, the grids were stained with 1% (w/v) phosphotungstate (pH 7.0) and examined with an electron microscope (Philips 201) at an accelerating voltage of 60 kV (Harel *et al.*, 1992a).

Isolation of fimbriae, PAGE and immunoblotting. Crude fimbrial extracts were prepared from bacteria grown on MD-1

plates as described previously (Harel *et al.*, 1992b). After boiling the fimbrial samples for 5 min in buffer [10 mM Tris/HCl (pH 7.8)] containing 4% (w/v) SDS, 0.01 ml β -mercaptoethanol, 0.2 ml glycerol and 0.002% bromophenol blue, the samples were run on slab gels as described previously (Harel *et al.*, 1992a). Western blotting (immunoblotting) was carried out according to the method of Towbin *et al.* (1979). Crude fimbrial extracts from wild-type *E. coli* 4787, *E. coli* HB101(pIVA4; cosmid positive for F165₂ expression), and HB101(pPIL110-51) were electrophoresed on SDS-polyacrylamide gels. Following transfer to nitrocellulose filters, the electrophoresed preparations were reacted with anti-F165 fimbrial complex and anti-F1C fimbriae sera to identify the fimbrial bands.

Preparation of minicells and analysis of plasmid-encoded proteins. Plasmid-encoded proteins were expressed in *E. coli* DS910 minicells, which were prepared by sucrose density centrifugation (Pugsley & Oudega, 1987). Purified minicells were labelled with 50 μ Ci (1.85 MBq) of [³⁵S]methionine (DuPont). After being labelled for 1 h at 37 °C, proteins from minicells were acetone-precipitated and resuspended in 100 μ l sample buffer (Labigne-Roussel *et al.*, 1984). Samples (10⁵ c.p.m.) were loaded on 12% (w/v) gels for SDS-PAGE. The gel was fixed, treated with Enhance (DuPont) and dried. It was then exposed on a XAR-5 film with an intensifying screen at -70 °C.

RESULTS AND DISCUSSION

Cloning of the F165₂ fimbriae determinant

Genomic DNA was isolated from the *E. coli* 4787 possessing the F165 fimbrial complex. After cleavage with *Sau*3A and a packaging procedure, 600 *E. coli* HB101 colonies were screened for the components of the F165 complex by the immunocolony detection technique. One of the recombinant clones harbouring a cosmid, designated pIVA4, that was found to react with F165 complex antiserum, was also *sfa*-positive by colony hybridization using an *sfa* probe derived as a 0.8 kb *Pst*I fragment from pILL1007. The expression by this clone of fimbriae reacting with F165 antiserum was confirmed by the isolation of fimbriae and by subsequent SDS-PAGE and immunoblotting with F165 antiserum. The absence of MRHA properties suggested the presence of *foc*-like, rather than *sfa*, coding genes.

The pIVA4 DNA was further cleaved with *Bam*HI, and suitable fragments were ligated into the *Bam*HI site of pACYC184. A recombinant plasmid, pYvan, conferred on strain HB101 expression of fimbriae as demonstrated by electron microscopy, but without specific haemagglutination (Fig. 1). Because of their immunological properties, and their absence of haemagglutination properties, the clones derived from the *sfa*-positive cosmid were thus described as *foc*-like clones. The structures could be identified as F165₂ fimbriae by immunogold labelling experiments using anti-F1C serum (Fig. 1).

Restriction map of pYvan and characterization of the F165₂ fimbriae

The recombinant plasmid pYvan consisted of the vector pACYC184 and a chromosomal insert of 13 kb. The physical map of pYvan was further determined by

digestion with different restriction enzymes. The results indicated that the *f165*₂ gene cluster is physically related to both *foc* and *sfa* gene clusters (data not shown) (van Die *et al.*, 1984; Hacker *et al.*, 1985, 1993; Ott *et al.*, 1986;

Fig. 1. Transmission electron micrograph of *E. coli* HB101(pYvan) labelled using the immunogold technique. Cells were incubated with antiserum raised against F1C fimbriae and then with protein A-gold (10 nm). Bar, 200 nm.

Fig. 2. Autoradiogram of 12% SDS-PAGE representing minicell analysis of DS910 carrying plasmids pYvan (lane A), pPIL110-51 (lane B) and pACYC184 (lane C). The dots represent the additional bands seen in lanes A and B, in comparison with lane C.

Fig. 3. Western blot of fimbrial subunits from different *E. coli* strains using (a) anti-F1C serum produced from clone pPIL110-51 and (b) anti-F165 serum produced from strain 4787. Lane A, HB101(pYvan); lane B, HB101(pPIL110-51); lane C, strain 4787.

Pawelzik *et al.*, 1988; Schmoll *et al.*, 1990). pYvan encodes at least five different proteins and showed a pattern similar to that of pPIL110-51 (which expresses fimbriae F1C) which had additional bands of 17, 20, 23, 30 and 100 kDa (Fig. 2, lanes A and B) in comparison with the vector pACYC184 alone (Fig. 2, lane C). The 17.2 kDa fimbrillin subunit molecule was not labelled since the mature protein does not contain methionine. Some of these proteins are probably involved in transport and biogenesis of F165₂, as already demonstrated for several other fimbriae (Tennent *et al.*, 1990).

Immunoblotting of the fimbrial extracts using anti-F165 complex serum revealed two bands, of 18.5 kDa and 17.2 kDa, in parent strain 4787, but only one band of 17.2 kDa in subclone HB101(pYvan) (Fig. 3). Moreover, antiserum directed against F1C fimbrial extract from HB101(pPIL110-51) recognizes only one band of 17.2 kDa in the fimbrial extracts from wild-type strain

4787, HB101(pYvan) and HB101(pPIL110-51) (Fig. 3). The second band of 18.5 kDa, visible in fimbrial extracts of *E. coli* 4787 examined with F165 complex antiserum, is the major protein subunit of F165₁, a Prs-like fimbria (Harel *et al.*, 1991; Maiti *et al.*, 1994). The size of F165₂ major fimbrial protein is similar to that of the major fimbrial proteins of the S/F1C fimbriae (16–17 kDa). The similarities between the latter subunit proteins have also been demonstrated by serological cross-reactions using anti-F1C serum (Pawelzik *et al.*, 1988).

Nucleotide sequence of *f165₂A* and *f165₂FGH*

The complete nucleotide sequence of the *f165₂A* gene was determined. This sequence contains a single ORF of 546 bp which encodes a protein of 180 amino acids (Fig. 4). A signal peptide of 24 amino acids is cleaved from the precursor protein to generate the mature F165₂A protein of 16.8 kDa (data not shown). The signal peptide is hydrophobic and has complete sequence similarity with that found in F1C fimbriae (van Die *et al.*, 1984). The *f165₂A* sequence showed an overall 98.9% identity with *focA* gene (data not shown).

Physical mapping of the *f165₂* operon showed that the three genes *f165₂F*, *G* and *H* were located at the 3' end of the operon. Thus, a fragment carrying the three genes was subcloned to generate the plasmid pF165₂FGH. An ORF for each of the *F* (528 nt), *G* (504 nt), and *H* (900 nt) genes has been identified. Sequences of *f165₂F*, *G* and *H* shared an overall 97.9% identity with those of *focF*, *G*, *H* (van Die *et al.*, 1991). The sequences *f165₂F/focF* and *sfaG* were found to be identical. Although *f165₂G* and *focG* were almost identical, they differed greatly from *sfaS*. There was only one silent difference between *focG* and *f165₂G*. Similarly, *f165₂H* and *focH* differed greatly from *sfaH*.

Codon usage for *f165₂A* is compatible with genes normally present in *E. coli* as reported for fimbriae by van

	1	10	20	30	40	50
F1652A	MK	LKFISMAVFS	ALTGLGVATNASAA	TTVNGGTVHFKGEVVNAACAVNTNSFDQT	VNLGQ	
FocA	---	---	---	---	---	---
SfaIIA	---	---	---	---	---	---
SfaA	---	---	---	---	---	---
	70	80	90	100	110	
F1652A	VR	SERLKV	DGAKSNFVGFTIELNDCDSQVSAGAGIVFSGPAV	TG	KTDVLALQSSAAGSA	
FocA	---	---	---	---	---	---
SfaIIA	---	---	---	---	---	---
SfaA	---	---	---	---	---	---
	130	140	150	160	170	
F1652A	TN	VG	QITDHTGKVVPLDGT	ASSTFTLT	DGTNKIPFQAVYYATGQATAGIANADATFKV	
FocA	---	---	---	---	---	---
SfaIIA	---	---	---	---	---	---
SfaA	---	---	---	---	---	---
	180					
F1652A	QYQ					
FocA	---					
SfaIIA	---					
SfaA	---					

Fig. 4. Comparison of the deduced amino acid sequence of the major fimbrial subunit protein F165₂A with that of FocA, SfaIIA and SfaA fimbriae. The GenBank accession number for amino acids sequences of FocA, SfaIIA and SfaA are M13053, S53211, M35273, respectively (van Die *et al.*, 1984; Hacker *et al.*, 1993; Schmoll *et al.*, 1987). The first amino acid of the mature protein is numbered +1. Blank spaces were introduced to obtain maximum similarity. Amino acid identities with the F165₂A sequence are indicated by hyphens.

(a)		1	10	20	30	40	50	60
F1652F		MVKDI	IKT	VT	FS	CML	AG	SM
FocF		-----	-----	-----	-----	-----	-----	-----
SfaIIIG		-----	-----	-----	-----	-----	-----	-----
SfaG		-----	-----	-----	-----	-----	-----	-----
		70	80	90	100	110	120	
F1652F		SRQ	FT	AAG	DT	TP	AS	VF
FocF		-----	-----	-----	-----	-----	-----	-----
SfaIIIG		-----	-----	-----	-----	-----	-----	-----
SfaG		-----	-----	-----	-----	-----	-----	-----
		130	140	150	160	170	176	
F1652F		LLD	KD	KH	EL	KIN	VP	TT
FocF		-----	-----	-----	-----	-----	-----	-----
SfaIIIG		-----	-----	-----	-----	-----	-----	-----
SfaG		-----	-----	-----	-----	-----	-----	-----
(b)		1	10	20	30	40	50	60
F1652G		MKL	KAI	IL	AT	GL	IN	CI
FocG		-----	-----	-----	-----	-----	-----	-----
SfaIIS		-----	-----	-----	-----	-----	-----	-----
SfaS		-----	-----	-----	-----	-----	-----	-----
		70	80	90	100	110	120	
F1652G		IAA	NS	T	S	Q	W	K
FocG		-----	-----	-----	-----	-----	-----	-----
SfaIIS		-----	-----	-----	-----	-----	-----	-----
SfaS		-----	-----	-----	-----	-----	-----	-----
		130	140	150	160	168		
F1652G		PLK	V	G	S	T	K	V
FocG		-----	-----	-----	-----	-----	-----	-----
SfaIIS		-----	-----	-----	-----	-----	-----	-----
SfaS		-----	-----	-----	-----	-----	-----	-----
(c)		1	10	20	30	40	50	60
F1652H		MAY	S	Q	P	S	F	A
FocH		-----	-----	-----	-----	-----	-----	-----
SfaIIH		-----	-----	-----	-----	-----	-----	-----
SfaH		-----	-----	-----	-----	-----	-----	-----
		70	80	90	100	110	120	
F1652H		QNY	D	L	R	V	R	Q
FocH		-----	-----	-----	-----	-----	-----	-----
SfaIIH		-----	-----	-----	-----	-----	-----	-----
SfaH		-----	-----	-----	-----	-----	-----	-----
		130	140	150	160	170	180	
F1652H		PAK	M	L	P	E	P	G
FocH		-----	-----	-----	-----	-----	-----	-----
SfaIIH		-----	-----	-----	-----	-----	-----	-----
SfaH		-----	-----	-----	-----	-----	-----	-----
		190	200	210	220	230	240	
F1652H		RV	S	S	N	N	V	K
FocH		-----	-----	-----	-----	-----	-----	-----
SfaIIH		-----	-----	-----	-----	-----	-----	-----
SfaH		-----	-----	-----	-----	-----	-----	-----
		250	260	270	280	290	300	
F1652H		GV	Q	L	S	D	N	A
FocH		-----	-----	-----	-----	-----	-----	-----
SfaIIH		-----	-----	-----	-----	-----	-----	-----
SfaH		-----	-----	-----	-----	-----	-----	-----

Fig. 5. Comparisons of the predicted amino acid sequences among F165₂FGH and other FocFGH/SfaGSH sequences. The GenBank accession number for amino acid sequences of FocFGH, SfaIIGSH, SfaGSH are MS68237, S53210, and X16664, respectively (van Die *et al.*, 1991; Schmoll *et al.*, 1989; Hacker *et al.*, 1993). (a) F165₂F/FocF/SfaIIIG/SfaG proteins and (b) F165₂G/FocG/SfaIIS/SfaS proteins and (c) F165₂H/FocH/SfaIIH/SfaH proteins. Blank spaces were introduced to obtain maximum similarity. Amino acid identities with the F165₂FGH sequences are indicated by hyphens.

Die *et al.* (1986). The GC contents of the *f165₂F*, *G* and *H* genes were 45, 41.0 and 51 mol % respectively.

Comparison of F165₂ major and minor subunit proteins with S/Foc subunit proteins

The deduced amino acid sequence of the mature F165₂A major fimbrial subunit gene was compared with the corresponding sequences of other S-like fimbriae (Fig. 4).

F165₂A protein was almost identical to the corresponding F1C protein and demonstrated less similarity to Sfa, SfaII and F1A (Klemm, 1984; van Die *et al.*, 1984; Schmoll *et al.*, 1987; Hacker *et al.*, 1993). At the amino acid level, only one change was observed in comparison to F1C, in the N-terminal region at position +1 (alanine for valine) (Fig. 4). As shown for many fimbriae, four regions could be distinguished in the F165₂A protein (Fig. 4): the N-terminal region, the Cys-Cys loop, a variable region, and

the C-terminal region. The N- and C-terminal regions of the major fimbrial protein subunits of the S/F1C family and F165₂ are very homologous and consist of mainly hydrophobic amino acids (Fig. 4). Most differences were observed in two regions, the Cys-Cys loop and the variable regions, which are probably responsible for the divergence of F165₂A and FocA from SfaA (Fig. 4) (van Die *et al.*, 1984, 1986; Schmoll *et al.*, 1987; Hacker *et al.*, 1993).

The deduced amino acid sequences of the three genes of the putative minor fimbrial subunits of F165₂ revealed three precursor proteins, F, G, and H, which have 175, 167 and 299 amino acids, respectively. Each of the three proteins possessed the typical amino terminal signal sequence which is hydrophobic (Fig. 5). The three mature proteins of 148, 145, and 276 amino acids, representing F165₂F, G, and H, respectively, had calculated molecular masses of 15.5, 15.1 and 29.5 kDa, respectively. Four cysteine residues were present in the F165₂F protein (Fig. 5). Both the G and H proteins contained two cysteine residues in the N-terminal half of the protein which may form an intramolecular disulphide bridge. The penultimate amino acid was tyrosine, as was reported for the other S- and P-related minor fimbrial proteins (van Die *et al.*, 1991; Schmoll *et al.*, 1989; Maiti *et al.*, 1994). As in P fimbriae, the various major and minor proteins of the S/Foc family are highly conserved in their N- and C-termini, which might reflect the involvement of these regions in fimbrial polymerization through subunit-subunit interaction (van Die *et al.*, 1987; Hultgren *et al.*, 1991; Hal Jones *et al.*, 1992).

F165₂F was identical to FocF and SfaG/SfaII G. Although F165₂G and FocG were identical except for two amino acids difference at positions 86 and 87, these proteins were quite different from SfaS and SfaIIS. Similarly F165₂H and FocH were identical but differed from SfaH and SfaIIH (Fig. 5). It has been shown that for the S fimbriae, the 15 kDa protein SfaS is the adhesin that binds to carbohydrate chains terminating with α -sialyl-(2-3)- β -D-Gal-R (Parkkinen *et al.*, 1986; Moch *et al.*, 1987; Morschhäuser *et al.*, 1990), whereas F1C and F165₂ fimbriae lack that specific adhesive property. S fimbriae and F1C fimbriae adhere to renal tubules. SfaG subunit was shown to contribute to the binding of S fimbriae to renal tubules. It is not clear which of the F1C subunits is responsible for the observed adhesive properties. Moreover, it has been suggested that SfaG acts as a linker molecule for the adhesin (SfaS) which could bind SfaS to fimbriae (Moch *et al.*, 1987). SfaG and SfaH were shown to be involved in modulation of fimbriation by Morschhäuser *et al.* (1990). We speculate that F165₂H or F165₂G may be involved in recognition of an unknown receptor.

Septicaemic *E. coli* strain 4787 expresses Prs-like and F1C-like fimbriae. Studies with uropathogenic *E. coli* strain KS71 (O4:K12:H⁻), whose fimbriae strongly resemble those of another uropathogenic *E. coli* strain, AD110, have revealed that individual cells express either F1C or P fimbriae (Rhen *et al.*, 1983; Nowicki *et al.*, 1984). These results suggest a mutual regulation of expression as in the

uropathogenic strain 536, for which cross-regulation of virulence gene clusters was demonstrated (Morschhäuser *et al.*, 1994). Strains 4787 and KS71 have different O and K antigens and originate from different species, the pig and the human, respectively. These findings suggest that the two isolates are not clonally related. In human UTI, type 1C fimbriae have been associated with groups O2, O6, O18 and O75, with capsular type K5, with mannose-resistant (both P and non-P) adhesins and with haemolysin production (Siitonen *et al.*, 1993).

In conclusion, we have cloned the *f165₂* operon, and sequenced the *f165₂* major fimbrial subunit gene and three minor fimbrial subunit genes. It was previously found that, in strain 4787, *f165₁* was a mosaic operon consisting of sequences related to *pap* and *prs*. We have now found that the *f165₂* operon seems to be conserved and highly related to the *foc* operon and belongs to the *S/foc* fimbrial family. The F165₂ major and minor subunits have the highest identity with the F1C major and minor fimbrial subunits. The contribution of F165₂ fimbriae to septicæmia in pigs needs to be investigated.

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