Presence and Characterization of Extraintestinal Pathogenic *Escherichia coli* Virulence Genes in F165-Positive *E. coli* Strains Isolated from Diseased Calves and Pigs

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The virulence genotype profile and presence of a pathogenicity island(s) (PAI) were studied in 18 strains of F165-positive *Escherichia coli* originally isolated from diseased calves or piglets. On the basis of their adhesion phenotypes and genotypes, these extraintestinal pathogenic strains were classified into three groups. The F165 fimbrial complex consists of at least two serologically and genetically distinct fimbriae: $F165_1$ and $F165_2$. $F165_1$ is encoded by the *foo* operon (*pap*-like), and $F165_2$ is encoded by *fot* (*sfa* related). Strains in group 1 were *foo* and *fot* positive, strains in group 2 were *foo* and *afa* positive, and strains in group 3 were *foo* positive only. The strains were tested for the presence of virulence genes found mainly in extraintestinal pathogenic *E. coli* (ExPEC) strains. Although all the strains were positive for the *papA* variant encoding F11 fimbriae *incD*, *traT*, and *papC*, the prevalence of virulence genes commonly found in PAIs associated with ExPEC strains was highly variable, with strains of group 2 harboring most of the virulence genes tested. *papG* allele III was detected in all strains in group 1 and in one strain in group 3. All other strains were negative for the known alleles encoding PapG adhesins. The association of virulence genes with tRNA genes was characterized in these strains by using pulsed-field gel electrophoresis and DNA hybridization. The insertion site of the *foo* operon was found at the *pheU* tRNA locus in 16 of the 18 strains and at the *selC* tRNA locus in the other 2 strains. Furthermore, 8 of the 18 strains harbored a high-pathogenicity island which was inserted in either the *asnT* or the *asnV/U* tRNA locus. These results suggest the presence of one or more PAIs in septicemic strains from animals and the association of the *foo* operon with at least one of these islands. F165-positive strains share certain virulence traits with ExPEC, and most of them are pathogenic in piglets, as tested in experimental infections.

Escherichia coli is a frequent cause of intestinal and extraintestinal diseases in humans and animals. Typical extraintestinal infections include urinary tract infections, newborn meningitis, polyserositis, and septicemia. All these groups of pathogenic *E. coli* strains have been called extraintestinal pathogenic *E. coli* (ExPEC). The recognized virulence factors of ExPEC include diverse adhesins (e.g., P fimbriae, S/F1C fimbriae, F165 fimbriae, Afa/Dr adhesins, and type 1 fimbriae), toxins (e.g., hemolysin, cytotoxic necrotizing factor, and cytolethal distending toxin), surface antigens (e.g., group II and group III capsules and lipopolysaccharide), invasins (e.g., an invasin responsible for invasion of brain endothelium [IbeA, also called Ibe10]), iron uptake systems (e.g., the aerobactin system), and secretion systems (e.g., type III secretion systems). These virulence factors facilitate colonization and invasion of the host, avoidance or disruption of host defense mechanisms, injury to host tissues, and/or stimulation of a noxious host inflammatory response (34, 50).

Fimbrial antigen complex F165, which exhibits mannose-resistant hemagglutination, is found mostly on *E. coli* strains isolated from piglets and calves with septicemia and/or diarrhea (19) and from humans with septicemia (11). Most F165-positive *E. coli* isolates are not enterotoxigenic or hemolytic; produce aerobactin; are resistant to the bactericidal effects of serum; do not produce verotoxin; and are negative for fimbrial antigens F4, F5, F41, and F6 (24). However, most F165-positive isolates, whether of intestinal or extraintestinal origin, induce septicemia and polyserositis but rarely induce diarrhea or significant enteric lesions in experimentally infected newborn piglets (17, 18). The F165 fimbrial complex consists of at least two serologically and genetically distinct fimbriae: $F165_1$ and $F165_2$ (23, 25). The $F165_1$A major fimbrial subunit of F165, which is encoded by the *foo* operon, is closely related to that of P fimbriae of serotype F11 but bears a class III G adhesin similar to the P-related (Prs) adhesin of F13 fimbriae, with specificity for the galactose-*N*-acetyl-α-(1-3)-galactose-*N*-acetyl (GalNac-GalNac) moiety (17, 40). $F165_2$ fimbriae, which are encoded by the *fot* operon, are closely related to F1C fimbriae (26). F165-positive strains possess many of the attributes of ExPEC strains that cause septicemia.

Pathogenicity islands (PAIs), which are large clusters of virulence genes in the bacterial chromosome, have been identi-

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fied in many different bacterial pathogens (22). The PAIs of uropathogenic (UPEC) human strains were the first to be described in *E. coli*. At least four PAIs are present in the genome of UPEC strain 536. PAI I$_{536}$, inserted next to the *selC* tRNA locus, and PAI II$_{536}$, inserted next to the *leuX* tRNA locus, respectively encode the hemolysin and the Prs fimbrial adhesin. On the other hand, PAI III$_{536}$ is inserted next to the *thrW* tRNA locus and encodes the S fimbrial adhesin (14). PAI IV$_{536}$ is inserted next to the *asnT* tRNA locus and carries the *fyuA* (ferrin yersiniabactin uptake) and *irp1* (iron-repressible protein) through *irp5* genes originally found in the high-pathogenicity islands (HPIs) of various *Yersinia* species (10). Two PAIs were described in UPEC strain J96 and encode the hemolysin and P or Prs fimbrial adhesins. They are PAI I$_{J96}$, which is inserted next to the *pheV* tRNA locus, and PAI II$_{J96}$, which also encodes cytotoxic necrotizing factor type 1 and which is inserted next to the phenylalanine-specific *pheR* (also known as *pheU*) tRNA locus (22).

The population structure of *E. coli*, as represented by the *E. coli* reference (ECOR) collection (46), is thought to be clonal, since it comprises four major clonal groups called A, B1, B2, and D (28). Most ExPEC isolates belong to ECOR group B2 and, to a lesser extent, ECOR group D (5). Recently, it was shown that ECOR group B2 and D strains more often carry certain virulence genes, including *hly*, *pap*, *sfa*, and *kps*, than strains from the other ECOR groups (8). These studies suggest that a cluster of *E. coli* strains acquired virulence genes by horizontal transfer, thus defining a highly virulent group. The link between the B2 phylogenetic group and virulence in Ex-PEC was recently confirmed in studies with experimentally infected mice (48). In this study we have found that F165-positive strains from pigs and calves share virulence genes found in ExPEC strains and that one or more PAIs are present. Certain virulence attributes found in F165 strains can be associated with pathogenicity, as tested in our model. Although F165-positive strains have been isolated from clinical cases, they belong to the groups, according to the phylogenetic scheme proposed by other investigators (13, 24, 28), that are less virulent than human ExPEC strains. Moreover, the ExPEC genes of animal F165-positive *E. coli* strains were characterized for their relationship with PAIs.

MATERIALS AND METHODS

Bacterial strains. The 18 F165-positive *E. coli* isolates used in this study were obtained from the *Escherichia coli* Laboratory of the Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada. These isolates were originally isolated from calves or piglets and were isolated either from the intestines of animals with septicemia or from extraintestinal tissues of animals with septicemia. All isolates were from different animals and farms. Other strains and/or plasmids used as controls for assay development included *E. coli* strains MG1655, 536 (*fyuA irp1 irp2*), CFT073, and J96 (*papA papC papG* alleles I and III *sfa/foc fimA kpsMT* III *hlyC cnf1*); *E. coli* O157:H7 (E-*hlyA espP etpD katP*); *Yersinia pseudotuberculosis* IP32637 (for detection of the FyuA protein); and *E. coli* strains IA2 (*papG* allele II), CS31A (*clpG f17c-A*), JM109/ pAH1010 (*nfa* allele I), pKT107 (*traT*), HB101/pILL 1194 (*afa-7*), and HB101/ pILL 1224 (*afa-8*). The strains were stored at −70°C in Luria broth plus 15% glycerol until they were ready for use. The *E. coli* strains were routinely grown overnight in Luria-Bertani (LB) broth with shaking at 37°C.

Detection of virulence genes and rapid phylogenetic analysis by PCR. The primers used for amplification of the virulence genes were derived from different sources or were designed from available nucleotide sequences (Table 1). All strains were tested for the presence of the three alleles of *papG* and 11 variants of *papA* by established specific PCR assays (30, 36). The virulence determinants

examined in this work were chosen because of their association with *E. coli* strains causing extraintestinal infections. The strains were screened for PCR products specific for genes encoding adhesins: P (*pap*), S/F1C (*sfa*), type 1 (*fimA*), F17c (*f17c-A*), and Afa/Dr (*afa*) adhesins; M blood group antigen-specific M fimbriae (*bma*); nonfimbrial adhesin type 1 (*nfa*); and Iha (*iha*) nonhemagglutinating adhesin (55). The toxin genes screened for were those encoding alpha-hemolysin (*hly*), *cnf*, and E-*hlyA* (53) and *cdtB* (cytolethal distending toxin) (34). The siderophore systems screened for included aerobactin (*iucD*), yersiniabactin (*fyuA*), and *iroN*$_{E. coli}$, a novel catechol siderophore receptor (33). The *cvaC* gene, which encodes colicin V (34), and the *iss* and *traT* genes, which encode proteins that increase resistance to serum (6, 12), were included in this study, as were other genes found in ExPEC, such as the gene for invasion of brain endothelium (*ibeA* or *ibe10*) (34) and capsular polysaccharide synthesis genes *kpsMT* II (e.g., K1, K5, and K12) and *kpsMT* III (e.g., K10 and K54) (34). Proteases encoding the *ompT* (outer membrane protein T), *tsh* (15), and *espP* (53) genes were also included in the study.

Bacterial DNA was released by the boiling method. PCR assays were carried out with the reagents and by the protocols supplied by the manufacturer (Pharmacia). The total reaction mixture volume was 50 μl, which contained 10 μl of supernatant from the boiled bacteria, the appropriate oligonucleotide primers at concentrations of 0.5 μM each, 200 μM (each) deoxynucleotide triphosphate, 5 μl of 10× PCR buffer (Pharmacia), and 2.5 U of *Taq* DNA polymerase (Pharmacia). The reactions with all the reaction mixtures included an initial denaturation at 94°C for 5 min and a final cycle of primer extension at 72°C for 7 min. The thermocycler reaction conditions for each primer pair were calculated on the basis of the annealing temperature and the length of the product size. PCR-amplified DNA was analyzed on 0.8 to 1% agarose gels by electrophoresis.

The phylogenetic group to which the *E. coli* strains belonged was determined by a PCR-based method, as described by Clermont et al. (13). Briefly, a two-step triplex PCR was performed directly with 3 μl of each of the bacterial lysates. The primer pairs used were ChuA.1-ChuA.2, YjaA.1-YjaA.2, and TspE4C2.1-TspE4C2.2 (Table 1). The PCR steps were as follows: denaturation for 4 min at 94°C; 30 cycles of 5 s at 94°C, 10 s at 59°C, and 30s at 72°C; and a final extension step of 5 min at 72°C. The data from the three amplifications resulted in assignment of the strains to phylogenetic groups as follows: *chuA* positive and *yjaA* positive, group B2; *chuA* positive and *yjaA* negative, group D; *chuA* negative and TspE4.C2 positive, group B1; *chuA* negative and TspE4.C2 negative, group A (13).

DNA probes specific for *pheU* tRNA, *thrW* tRNA, *selC* tRNA, *leuX* tRNA, *metV* tRNA, *afaE-8*, *fimA*, *papC*, *sfaDE*, *f17c-A*, *iucD*, *irp2*, *fyuA*, *traT*, *iss*, *cvaC*, *hlyC*, *ehlyA*, *cnf*, *cdt3*, and *cdt4* were generated by PCR with suitable primer pairs (Table 1). Following amplification, the PCR products were run on a 1% agarose gel. Appropriate fragments were cut from the gel, concentrated by ethanol precipitation, and/or purified with the Qiaquick PCR purification kit (Qiagen, Inc. Chatsworth, Calif.) and then radiolabeled with [α-^{32}P]dCTP by using a random priming kit for labeling of oligonucleotides (Pharmacia LKB Biotechnology Inc., Baie d'Urfé, Québec, Canada), according to the instructions of the manufacturer.

Detection of FyuA by immunoblotting. The positive control bacterial strain used for PCR amplification of *fyuA* and for detection of the FyuA protein was *Y. pseudotuberculosis* IP32637 (kindly provided by Elisabeth Carniel). For this purpose, bacteria were grown overnight in LB broth with shaking at 28°C (*Yersinia*) or 37°C (*E. coli*) (54). Iron-restricted medium was prepared by the addition of 2,2′-dipyridyl to a final concentration of 50 μM, and iron-rich medium was prepared by adding 150 μM FeCl$_3$. The same quantity of bacteria of each strain was sonicated, the cell debris was removed, and the supernatant was centrifuged at 20,000 × g for 1 h at 4°C. The pellet fraction was incubated for 1 h in 1% sodium dodecyl sulfate (SDS) at room temperature and centrifuged again (at 20,000 × g for 1 h). The supernatant was dialyzed against water and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12% polyacrylamide gel. The gel was electroblotted onto a nitrocellulose membrane, and FyuA was detected with anti-FyuA antiserum kindly provided by J. Heesemann. Goat anti-rabbit antibody conjugated to horseradish peroxidase was used as the secondary antibody, and the reaction was developed with hydrogen peroxide and 4-chloro-1-naphthol as the substrate.

PFGE and Southern hybridization. The pulsed-field gel electrophoresis (PFGE) and Southern hybridization procedure used was a modified method of Gautom (21). The bacteria were grown overnight at 37°C in LB broth and centrifuged at 1,500 × g for 15 min at 4°C. The cells were washed in SE (75 mM NaCl, 25 mM EDTA [pH 7.5]) and centrifuged, and the optical density of the cells at a wavelength of 600 nm was adjusted to between 1.60 and 1.80 in cold TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). For each bacterial strain, 500 μl of suspension was added to 500 μl of prewarmed (60°C) 1.5% low-melting-

TABLE 1. PCR primers and conditions used in this study

Gene	Primer pair (5′-3′)[a]	Annealing temp (°C)	Predicted size (bp)	Reference or source
papC	F GACGGCTGTACTGCAGGGTGTGGCG R. ATATCCTTTCTGCAGGGATGCAATA	60	328	39
sfaDE	F CGGAGGAGTAATTACAAACCTGGCA R. CTCCGGAGAACTGGGTGCATCTTAC	60	410	39
afaBC III	F GCTGGGCAGCAAACTGATAACTCTC R CATCAAGCTGTTTGTTCGTCCGCCG	60	793	39
afaE-7	F GCTAAATCAACTGTTGATGTT R. GGACAATCCAAATGGCGAATTA	65	618	38
afaE-8	F CTAACTTGCCATGCTGTGACAGTA R. TTATCCCCTGCGTAGTTGTGAATC	65	302	38
clpG	F. GGGCGCTTCTCTCCTTCAAC R CGCCCTAATTGCTGGCGAC	55	402	4
20K (*f17c-A*)	F GCAGAAAATTCAATTTATCCTTGG R CTGATAAGCGATGGTGTAATTAAC	55	537	4
fimA	fimA05 GTTGATCAAACCGTTCAG fimA16 AATAACGCGCCTGGAACG	55	331	42
bmaE	F ATGGCGCTAACTTGCCATGCTG R AGGGGGACATATAGCCCCCTTC	60	507	34
nfaE	F GCTTACTGATTCTGGGATGGA R CGGTGGCCGAGTCATATGCCA	60	559	34
iha	F. CTGGCGGAGGCTCTGAGATCA R TCCTTAAGCTCCCGCGGCTGA	55	827	33
iucD	F AAGTGTCGATTTTATTGGTGTA R CCATCCGATGTCAGTTTTCTG	60	760	27
fyuA	FyuA. 1A. GGCGGCGTGCGCTTCTCGCA FyuArp CGCAGTAGGCACGATGTTGTA	60	209	1
irp1	F GCGATGTTTAACCCCGATT R TGCCTGGAAACCCTGAGACT	55	1,691	1
irp2	Irp2. 15: GTTGCTGTCCATCAAGCACG Irp2. 18 GCCGGAAAGCCTGGCCTTTA	60	1,243	1
iroN	F AAGTCAAAGCAGGGGTTGCCCG R GACGCCGACATTAAGACGCAG	55	665	33
kpsMT II	F GCGCATTTGCTGATACTGTTG R CATCCAGACGATAAGCATGAGCA	60	272	34
kpsMT III	F TCCTCTTGCTACTATTCCCCCT R AGGCGTATCCATCCCTCCTAAC	60	392	34
traT	F GGTGTGGTGCGATGAGCACAG R CACGGTTCAGCCATCCCTGAG	60	290	34
iss	F TCACATAGGATTCTGCCG R AGAAATCAAAAGGTGGCC	50	607	This study
cvaC	F CACACACAAACGGGAGCTGTT R CTTCCCGCAGCATAGTTCCAT	55	680	34
ompT	ompt-f ATCTAGCCGAAGAAGGAGGC omp-r CCCGGGTCATAGTGTTCATC	60	559	32
espP	esp-A AAACAGCAGGCACTTGAACG esp-B GGAGTCGTCAGTCAGTAGAT	56	1,830	9

Continued on following page

TABLE 1—*Continued*

Gene	Primer pair (5'-3')[a]	Annealing temp (°C)	Predicted size (bp)	Reference or source
ibe10	F AGGCAGGTGTGCGCCGCGTAC R TGGTGCTCCGGCAAACCATGC	60	170	34
hlyC	F AGGTTCTTGGGCATGTATCCT R TTGCTTTGCAGACTGCAGTGT	60	556	5
E-*hlyA*	F GGTGCAGCAGAAAAAGTTGTAG R TCTCGCCTGATAGTGTTTGGTA	57	1,551	52
cnf	F TTATATAGTCGTCAAGATGGA R CACTAAGCTTTACAATATTGAC	50	636	47
tsh	tsh-1 GGTGGTGCACTGGAGTGG tsh-2 AGTCCAGCGTGATAGTGG	53	640	15
chuA	F GACGAACCAACGGTCAGGAT R TGCCGCCAGTACCAAAGACA	59	279	13
yjaA	F TGAAGTGTCAGGAGACGCTG R ATGGAGAATGCGTTCCTCAAC	59	211	13
TspE4 C2	F GAGTAATGTCGGGGCATTCA R CGCGCCAACAAAGTATTACG	59	152	13
int	F TCCCTTACCGACGCAAAAATCC R TGCTTCCAGATAATCCGACCAC	58	1,203	37
asnT-int	IntSB ATCGCTTTGCGGGCTTCTAGGT AsnTSB GAACGGCGGACTGTTAAT	60	1,393	1
asnV-int	F GACAGCAAACAAACAAAAA R TGCTTCCAGATAATCCGACCAC	60	1,500	37
asnU-int	F TTTCGCTGTTAAGATGTGCC R TGCTTCCAGATAATCCGACCAC	60	1,500	37
IS*100*	F ATTGATCCACCGTTTTACTC R CGAACGAAAGCATGAAACAA	60	963	1
selC tRNA	F GAGCGAATATTCCGATATCTGGTT R CCTGCAAATAAACACGGCGCAT	60	527	45
phcU tRNA	F TTC AGA AAA TCT CAT CAG TCG C R CAG AAA CAC AGA AAA GAA GCG A	60	475	This study
thrW tRNA	F TGT TTA CGT TAA CGC CTC TAC G R TGA GCT AAT TTG TTC GAG CTT T	60	586	This study
leuX tRNA	F TGC TGA AAA TTT CAG CAC TTA G R ATT TTT TGC TTT CCC TCA TAA C	55	520	This study
metV tRNA	F GGT AAA AAA AAG GTT GCA TGA A R TAA AAA TCA AGTTGA ACA GGC C	55	628	This study
afaE-8 and *pheV*	F GATTCACAACTACGCAGGGG R ATTTGATTGACGAGACGAGGCGAA	65	1,800	38
afaE-8 and *pheU*	F GATTCACAACTACGCAGGGG R CCGAACTCAACCAGATTCTCCCC	65	1,850	38

[a] F, forward; R, reverse.

point agarose (Sigma), and the mixture was immediately poured into a block former (plug mold). After solidification, the plugs were incubated overnight at 50°C in 1 ml of lysis buffer (1% [wt/vol] *N*-laurylsarcosine, 0.5 M EDTA [pH 9.5]) supplemented with 1 mg of proteinase K per ml. To inactivate the proteinase K, the plugs were then washed three times (each time 1 h) in TE buffer supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) at room temperature and were run for 45 min at 60 V in 0.5× TBE buffer (44.5 mM Tris-borate, 12.5 mM EDTA [pH 8.3]). This premigration step removed degraded or extrachromosomal DNA from the gel and, thus, strongly reduced the smear background in PFGE (7). Macrorestriction of genomic DNA embedded in agarose blocks with restriction enzymes *Xba*I and *Sfi*I was done as described by Birren and Lai (7). The DNA was separated on an AutoBase machine (Mandel Scientific Com-

TABLE 2. Virulence genotypes of 18 F165-positive *E. coli* strains isolated from swine and cattle[a]

Strain	Group	Adhesins								Iron uptake					Serum resistance				Proteases	
		papC	*sfaDE*	*afaE-8*	*clpG*	20K	*fimA*	*bmaE*	*iha*	*iucD*	*fyuA*	*irp1*	*irp2*	*iroN*	*kpsMT* III	*traT*	*iss*	*cvaC*	*ompT*	*espP*
5131	1	+	+	−	−	−	+	−	−	+	−	−	−	+	−	+	+	+	−	−
4787	1	+	+	−	−	−	+	−	−	+	−	−	−	+	−	+	+	+	+	−
6389	1	+	+	−	−	−	+	−	−	+	−	−	−	+	−	+	+	−	+	−
1776	1	+	+	−	−	−	+	−	−	+	−	−	−	+	−	+	−	−	−	−
393	1	+	+	−	−	−	+	−	−	+	−	−	−	+	−	+	−	−	+	−
2325	2	+	−	+	+	−	+	+	+	+	+	+	+	−	−	+	−	−	+	−
3292	2	+	−	+	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	−
7867	2	+	−	+	−	−	+	+	−	+	+	+	+	+	−	+	+	+	+	−
3863	2	+	−	+	−	−	+	+	−	+	+	+	+	+	−	+	+	+	+	−
1401	2	+	−	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+
3984	2	+	−	+	−	−	+	+	−	+	+	−	−	+	−	+	+	−	+	−
215	2	+	−	+	+	−	+	+	−	+	−	−	−	−	+	+	−	−	+	+
2313	3	+	−	−	−	−	+	−	−	+	−	−	−	−	−	+	−	−	−	−
2878	3	+	−	−	+	−	+	−	−	+	−	−	−	−	−	+	−	−	−	+
1195	3	+	−	−	−	−	+	−	−	+	+	+	+	−	−	+	−	+	+	−
1616	3	+	−	−	−	−	+	−	−	+	+	−	+	+	−	+	+	−	−	−
3373	3	+	−	−	−	−	+	−	−	+	+	+	+	−	−	+	−	−	+	−
3719	3	+	−	−	−	−	+	−	−	+	−	−	−	+	−	+	+	+	+	−

[a] The genotypes shown are for virulence factors detected by PCR and/or probe hybridization. + and −, presence and absence of a gene, respectively. *papC,* gene responsible for pilus assembly that is found in the central region of *pap* operon; *sfal/focDE,* genes found in the central region of *sfa* (S fimbriae) and *foc* (F1C fimbriae) operons; *afaE-8,* afimbrial adhesin gene, *clpG,* gene for structural subunit of CS31A surface antigen; 20K, F17C fimbria; *fimA,* gene for type 1 fimbrial major subunit; *bmaE,* gene for blood group M-specific adhesin; *iha,* IrgA (iron-regulated gene A of *Vibrio cholerae*) homologue adhesin; *iucD,* gene that encodes a membrane-bound enzyme synthesizing N_6-hydroxylysine, the first product of the aerobactin biosynthesis pathway; *iroN,* gene for a novel catechole siderophore receptor; *fyuA,* gene for *Yersinia* siderophore receptor (ferric yersiniabactin uptake); *irp1* and *irp2,* genes for iron-repressible high-molecular-weight proteins involved in the production of yersiniabactin; *kpsMT* allele III, gene for group III capsular polysaccharide synthesis (e.g., K3, K10, and K54); *traT,* gene for surface exclusion, serum survival (outer membrane protein); *iss,* gene for increased serum survival; *cvaC,* colicin V, located on conjugative plasmids (*traT, iss,* and antimicrobial resistance); *ompT,* gene for outer membrane protein T (protease); *espP,* gene for *E. coli* serine protease.

pany Ltd.), which uses the principle of zero integrated field electrophoresis, a variation of the field inversion gel electrophoresis configuration, which gives sharper bands and minimum band inversion (7). PFGE was performed with a 0.8% agarose gel in 1× TBE buffer at room temperature without cooling or circulation of buffer. PFGE Marker I (Roche Diagnostics Corporation, Laval, Quebec, Canada), a bacteriophage lambda DNA digest consisting of a ladder (20 bands) of increasing size from 48.5 kb (monomer) to approximately 1,000 kb (20-mer), was included as a DNA size standard. The digested genomic DNA separated in agarose gels was transferred to positively charged nylon membranes (Immobilon-Ny$^+$; Millipore Corporation, Bedford, Mass.) in accordance with the instructions of the manufacturer and hybridized under stringent conditions as described by Sambrook et al. (51).

Determination of plasmid profiles by nuclease S1 treatment and PFGE. To determine the molecular sizes of the bacterial high-molecular-weight plasmids accurately, plasmid profiles were determined by using nuclease S1 treatment followed by PFGE, as described elsewhere (3). Half of each plug was rinsed with 200 µl of 1× nuclease S1 buffer (50 mM NaCl, 30 mM sodium acetate [pH 4.5], 5 mM ZnSO$_4$) at room temperature for 20 min before being incubated with 1 U of *Aspergillus oryzae* nuclease S1 (Sigma, St. Louis, Mo.) in 200 µl of 1× nuclease S1 buffer at 37°C for 45 min. The reaction was stopped by adding 10 µl of 0.5 M EDTA (pH 8.0). Electrophoresis was performed on a CHEF DR II PFGE apparatus (Bio-Rad Laboratories, Hercules, Calif.) in 0.5× TBE buffer at 14°C. The gels were then run at pulse ramp times ranging from 2 to 35 s for 20 h at a constant voltage of 200 V. For size determination, a 48.5-kb bacteriophage lambda DNA ladder (Roche Diagnostics Corporation, Laval, Quebec, Canada) was used as a molecular size marker. After electrophoresis, the gels were stained with ethidium bromide and were photographed with an AlphaImager 2000 camera (Alpha Innotech Corporation, San Leandro, Calif.).

Experimental infections. Strains were tested for pathogenicity by experimental infection of colostrum-deprived newborn piglets. When the piglets were 2 days old, they were intragastrically given 1 ml of an overnight culture in tryptic soy broth containing approximately 10^9 CFU of *E. coli* ml^{-1} diluted in 20 ml of 0.1% peptone water. The pigs were observed for clinical signs and were necropsied when moribund or at 4 days after inoculation if no clinical signs were observed. Bacteriological and pathological examinations were done as described previously (17, 18).

RESULTS

Virulence factor profiles including profiles for *papG* and *papA* alleles. A total of 18 F165-positive septicemic *E. coli* strains from animals were originally selected from among a group of isolates found to be positive with probes for *pap*, *sfa*, or *afa* on colony hybridization. Only single copies of the *pap*-, *sfa*-, and *afa*-related sequences were found among these isolates (23, 41). These isolates had been divided into three groups on the basis of their adhesion phenotypes and genotypes. Isolates in group 1 were *pap* and *sfa* positive, isolates in group 2 were *pap* and *afa* positive, and isolates in group 3 were *pap* positive only (Table 2). Among 11 recognized variants of *papA,* the major pilin gene of *E. coli* P fimbriae, which are termed F7-1, F7-2, and F8 to F16 (36), only the F11 *papA* variant was detected in all 18 strains. The PapG adhesin occurs in three known molecular variants, encoded by alleles I to III of the corresponding *papG* gene (29). Allele III was detected in all strains in group 1, all of which belonged to serogroup O115, and in one strain in group 3, which belonged to serogroup O9. All of these strains were of porcine origin. Although the strains in groups 2 and 3 were positive for the *pap* operon, they were negative for all three of the *papG* alleles tested. All strains were positive for *iucD*, *traT*, and *papC* and were negative for cytotoxin genes commonly found in PAIs associated with ExPEC strains (*cnf*, *hlyC*). All strains were negative for *afaE-3, afaE-7, nfaE, cdt3, cdt4,* E-*hlyA, ibeA* or *ibe10, kpsMT* II, and *tsh*.

Strains in group 1 (*pap* and *sfa* positive) were also positive for *fimA* and *iroN*. Some strains were also positive for *iss, cvaC,*
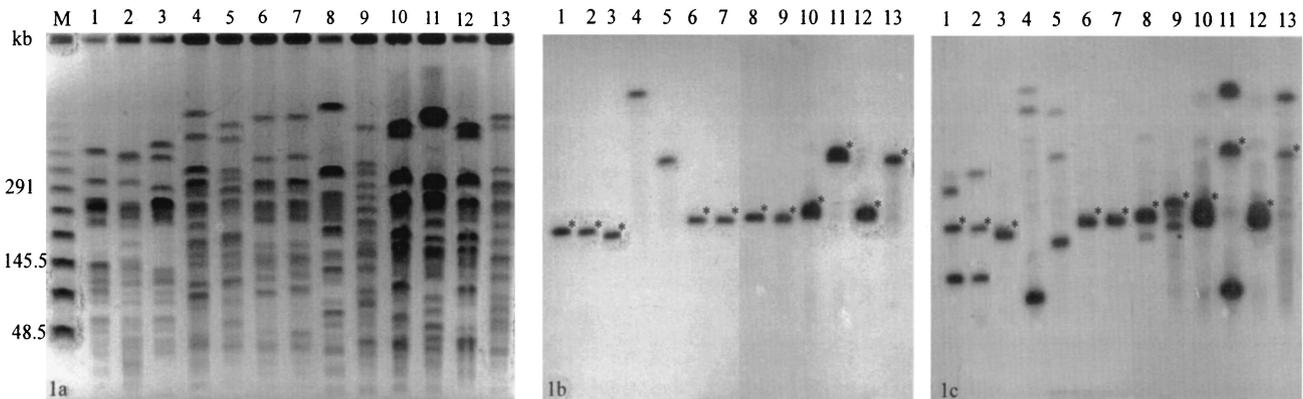
FIG. 1. (a) Macrorestriction patterns of *Xba*I-digested genomic DNA from F165-positive strains analyzed by PFGE. (b and c) Southern blot of F165-positive strains digested with *Xba*I and separated by PFGE (b, hybridization with a *pheU* tRNA gene-specific probe; c, hybridization with a *papC*-specific probe). The asterisks on the right sides of certain fragments indicate the fragments that hybridized with both probes. Lanes: M, bacteriophage λ (PFGE ladder); 1, 5131; 2, 4787; 3, 1776; 4, 2325; 5, 3292; 6, 7867; 7, 3863; 8, 215; 9, 2878; 10, 1195; 11, 1616; 12, 3373; and 13, 3719.

and *ompT* (Table 2). Strains in group 2 (*pap* and *afa* positive) harbored the chromosomal *afaE-8* operon, which is specific for human and bovine septicemia and which seems to be very similar to the M agglutinin of uropathogenic strains from humans (38). Strains in group 2 possessed most of the virulence factors for which tests were conducted. They were all positive for *bmaE*, *iucD*, *traT*, and *ompT*. Three strains in this group (strains 2325, 1401, and 215) were positive for *clpG*, which encodes capsule-like antigen CS31A (4). Two strains (strains 3292 and 1401) were positive for the F17c (also known as 20K) fimbria. Four strains were positive for all the iron uptake systems for which tests were conducted. Four strains (strains 3292, 7867, 3863, and 3984) were positive for *iss*, and three strains (strains 3292, 7867, and 3863) were positive for *cvaC* (colicin V), which is carried by conjugative plasmids. Strain 215 was the only strain in group 2 that was positive for *kpsMT* III and negative for *fimA*. Although *iucD* and *traT* were found in strains in group 3 (*pap* positive), the presence of virulence genes was more variable. Strain 2878 was the only strain in this group that was positive for *clpG*. Interestingly, *espP* (*E. coli* serine protease), which is found in most enterohemorrhagic strains (9), was detected in three bovine F165-positive strains in groups 2 and 3 (Table 2).

Locations of PAIs encoding fimbrial operons. Many of the PAIs identified are associated with tRNA loci (22). To study the regions containing the *foo* (*pap*-like), *fot* (*sfa*-related), and *afaE-8* operons, DNA from PFGE separations was transferred to membranes and was hybridized with different tRNA gene-specific probes. For most strains (16 of 18 F165-positive strains), the band that hybridized with the probe for the *foo* operon cohybridized with the *pheU* tRNA gene-specific probe. This suggests that for these strains the insertion site of the putative PAIs encompassing the *foo* operon may be within the *pheU* locus. In two other strains the band that hybridized with the probe for the *foo* operon cohybridized with the *selC* tRNA gene-specific probe. This suggests that for these two strains, the insertion site of the PAIs encompasing the *foo* operon may be within the *selC* locus. To establish whether the virulence genes are linked in the same segment of the chromosome,

suggesting their linkage in a PAI, the PFGE bands generated by digestion with two restriction enzymes (*Xba*I and *Sfi*I) were hybridized with probes specific for *papC*, *sfaDE*, *afaE-8*, *fimA*, and *F17c-A*. None of these genes were located on a single restriction fragment, regardless of the restriction enzyme used, suggesting that either there are unknown virulence genes within these putative PAIs or they only contain fimbrial operons as a sole virulence factor.

Sets of PCR primers that could amplify the intact regions containing the tRNA genes were used to confirm that some fimbrial operons in F165-positive strains were inserted within or near tRNA loci (Table 1). We investigated whether the insertion sites of the fimbrial operons in the F165-positive isolates resided within the *pheU*, *thrW*, *selC*, *leuX*, and *metV* tRNA loci (data not shown). Our results indicated that, for most strains, the *pheU* locus was not amplified and that for two strains in group 3 the *selC* locus was not amplified. This suggests that in these isolates these tRNA loci were rearranged or disrupted. Different primer pairs covering the phenylalanine-specific tRNA genes (*pheU* and *pheV*) and the *afa-8* gene were used to determine the insertion site of the *afa-8* PAI (Table 1). In contrast to a recent study (38) on the *afa-8* PAI, the insertion site of this *afa-8* PAI in our group 2 strains was not detected in any of the *phe* tRNA loci.

For 16 of 18 strains, the *pheU* tRNA gene-specific probe hybridized with the fragment that contained *foo*, which is similar to PAI II$_{J96}$, which is also inserted next to the *pheU* locus, at 94 min of the *E. coli* K-12 chromosomal map (22) (Fig. 1). Furthermore, the *pheU* tRNA gene-specific probe did not hybridize with the fragment containing the *afa-8* operon. For only two group 2 strains (strains 2325 and 3292), the *selC* tRNA gene-specific probe hybridized with the fragment that contains the *foo* operon, which is similar to PAI I$_{536}$ (22). Hybridization with *foo* (*pap*-like) and *fot* (*sfa*-related) gene-specific probes for strains in group 1 showed that these two operons are not physically linked. Although *thrW* tRNA was disrupted in all strains in group 1 that were positive for both *foo* and *fot*, the *thrW* tRNA gene-specific probe did not hybridize with the fragment containing the *foo* or *fot* operon. This is different

from the finding for *E. coli* 536, in which an *sfa* operon homologous to *fot* was inserted next to *thrW* tRNA (PAI III$_{536}$) (14). For the group 2 strains, hybridization with *foo* and *afaE-8* gene-specific probes suggested that these two operons are not on the same fragment.

The PFGE patterns, which were heterogeneous among the different strains, were similar within the particular groups. PFGE analysis demonstrated that the most closely related strains were within the same group but were also of the same origin.

Presence of HPI in F165-positive strains. The HPI-specific genes (*irp2*, *irp1*, and *fyuA*) were found in 8 of 18 strains (only 7 of the 8 strains had all three genes); 5 of these strains belonged to group 2, and 3 of these strains belonged to group 3 (Table 2). Strain 1616 was positive for both *irp2* and *fyuA* but was negative for *irp1*. In *Yersinia* spp. and *E. coli*, the site of HPI integration into the bacterial chromosome shares homology with the P4 bacteriophage attachment site and is located within an *asn* tRNA locus (10). To determine whether the HPIs of these strains were bordered by an *asn* tRNA gene locus, a set of primers whose sequences covered a portion of the *int* and *asn* genes was used (Table 1). The insertion site of the HPI in six of eight strains was next to the *asnT* tRNA gene, as demonstrated by PCR with primer pairs specific for *asnT* and *int*. In agreement with a recent report (1), the size of the PCR product was smaller than that in *Y. pseudotuberculosis* (1,100 bp instead of the expected 1,393 bp) in two strains (strains 2325 and 3292), suggesting that a deletion of approximately 300 bp had taken place in this region. Interestingly, in one strain (strain 1401), amplification by PCR produced a band of the expected size and two other smaller bands (1,200 and 1,100 bp). Two strains (strains 1195 and 1616) did not yield any amplification product with three different *asn*-specific primers (specific for *asnT*, *asnV*, and *asnU*) or the *int*-specific primer. All of these strains harboring HPIs were positive for IS*100*, as demonstrated by use of specific primers.

Certain *E. coli* strains (up to 3% of *irp2*-positive strains) carry a truncated *fyuA*-*irp* gene cluster, with deletions proceeding from *fyuA* or from both *fyuA* and *irp1* to the 3′ end of the HPI. Such spontaneous deletions involve only the 3′ part of the *fyuA*-*irp* gene cluster and abrogate expression of the yersiniabactin receptor (54). In order to analyze the expression of *fyuA* in the eight strains carrying the *Yersinia* HPI, immunoblotting of outer membrane proteins was performed. One specific band of about 67 kDa for FyuA was detected in seven of the eight HPI-positive strains (data not shown).

Phylogenetic grouping results. Phylogenetic analysis studies have suggested that *E. coli* is composed of four main phylogenetic groups (groups A, B1, B2, and D) and that virulent ExPEC strains mainly belong to groups B2 and D, whereas commensal strains belong to groups A and B1. Recently, Clermont et al. (13) described a simple and rapid phylogenetic grouping technique based on a triplex PCR. The results of the method, which uses a combination of two genes (*chuA* and *yjaA*) and an anonymous DNA fragment, showed a good correlation with those of reference methods (13). Examination of the strains by the triplex PCR method demonstrated that the strains in group 1, all of which were O115 and positive for *foo* and *fot*, belong to phylogenetic group B1, whereas all the strains in groups 2 and 3 belong to phylogenetic group A.

High-molecular-mass plasmid profiles. In a previous study (24) it was shown that F165-positive strains carried several low-molecular-mass plasmids. In order to study the high-molecular-mass plasmids in 18 F165-positive strains, plasmid profiles were determined by using nuclease S1 treatment followed by PFGE. We identified plasmids with molecular masses ranging from 10 to 245 kb. Most of the strains that were positive by hybridization with probes specific for the *iucD*, *traT*, and *iss* genes harbored at least two high-molecular-mass plasmids of 90 to 245 kb. These plasmids were more frequently found in group 2 strains than in group 1 and 3 strains (Fig. 2.).

Pathogenicities of strains in pigs. The virulence of the F165-positive group 1 strains in piglets was high (Table 3). All O115 strains caused 100% mortality in pigs within 48 h of inoculation, with manifestation of gross lesions of polyserositis on necropsy. Pathogenicity was variable in strains of groups 2 and 3 (Table 3). Of four group 2 strains tested, three O11 or O9 strains caused 100% mortality in pigs within 48 h of inoculation, with manifestation of gross lesions of polyserositis on necropsy, whereas an O101 strain was nonpathogenic. Of three group 3 strains tested, an O9 strain caused mortality in pigs, whereas two O15 strains were nonpathogenic. All HPI-positive strains tested were pathogenic in piglets. On the other hand, the HPI-negative strains in group 1 tested were pathogenic, whereas those in groups 2 and 3 were less pathogenic or nonpathogenic in piglets. The strains in group 1 belong to phylogenetic group B1, whereas all strains in groups 2 and 3 belong to phylogenetic group A. No obvious relationship between the presence of the genes and pathogenicity in piglets was observed for any of the other virulence determinants.

DISCUSSION

We have previously defined three phenotypic and genotypic groups among F165-positive animal *E. coli* strains on the basis of the expression of three different fimbrial operons. Strains in group 1 were positive for *foo* (*pap*-like) and *fot* (*sfa*-related) genes, and strains in group 2 or 3 were *foo* and *afa* positive and *foo* positive only, respectively (41). The results of this study suggest the presence of one or more PAIs (*foo*, *fot*, *afaE-8*, HPI) in septicemic strains isolated from animals. Moreover, the tRNA gene locus associated with some PAIs was also determined. The P-like operons were located near the *pheU* or *selC* tRNA gene and HPI sequences near the *asnT* tRNA gene. The insertion site of the HPI in six of eight strains was next to the *asnT* tRNA gene. The insertion site of *afa-8* PAI in group II strains was not detected in any of the *phe* tRNA loci, which is in contrast to the findings in a recent study by Lalioui and Le Bouguenec (38) on the *afa-8* PAI. The insertion site of *fot*, which belongs to the Sfa/F1C family was not next to *thrW* tRNA, unlike the *sfa* operon in *E. coli* 536.

The linkage of the *foo* operon to the *pheU* tRNA gene in 16 strains and to the *selC* tRNA gene in 2 others, the low G+C content of this operon (41 to 43%, compared to a 50.8% content in this operon of *E. coli* K-12 [data not shown]), and its presence in pathogenic strains indicates that this operon is part of a PAI. Moreover, the F165$_1$ major fimbrial subunit (encoded by the *foo* operon) is closely related to that of P fimbria serotype F11 but bears a class III G adhesin similar to the Prs adhesin of F13 fimbriae (40) and may represent a new member
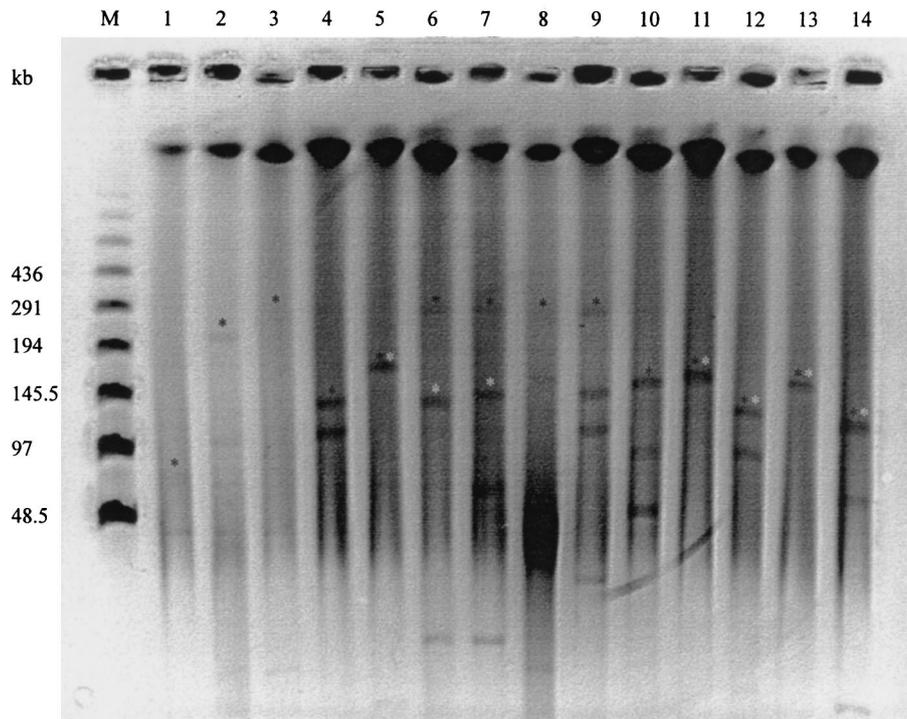
FIG. 2. Plasmid profiles of 14 F165-positive isolates, determined by nuclease S1 treatment followed by PFGE analysis. Black asterisks, plasmid bands hybridizing with the *traT*-specific probe; white asterisks, plasmid bands hybridizing with the *cvaC*-specific probe. Lanes: M, bacteriophage λ (PFGE ladder); 1, 5131; 2, 4787; 3, 1776; 4, 2325; 5, 3292; 6, 7867; 7, 3863; 8, 1401; 9, 215; 10, 2878; 11, 1195; 12, 1616; 13, 3373; and 14, 3719.

of the family of P fimbriae. Although all F165-positive strains possessed *papC*, only six, all being of porcine origin, had a PCR-detectable *papG* allele corresponding to *papG* allele III. The fact that most porcine strains were positive for allele III whereas all bovine strains were negative for all three known alleles could suggest that there are unknown *papG* alleles among these isolates. The observed predominance of *papG* allele III among the porcine isolates (six of nine) in the present study is consistent with the previously demonstrated predominance of *papG* allele III in canine fecal *E. coli* isolates (35). The *papG* allele III is also the most prevalent *papG* allele among *E. coli* isolates from humans with cystitis and in some reports is prevalent among isolates from patients with bacteremia and pyelonephritis (29, 31). Among the 11 PapA variants tested, only the F11 *papA* allele was detected in all 18 strains. In addition to the F165 fimbria, which was detected in the strains tested in this study, which were isolated from pigs and calves with septicemia, interestingly, F11 is the predominant serotype of P fimbriae expressed by *E. coli* strains implicated in avian colisepticemia (16). This suggests that F11 fimbriae are frequently expressed by pathogenic *E. coli* strains isolated from animals with septicemia. Marklund and coworkers (43) have proposed that *E. coli* acquired the *pap* locus after the speciation of *E. coli* and have suggested that the different *pap* genes could have been acquired by horizontal gene transfer. Moreover, they proposed that the recent genetic exchanges involving the entire fimbrial gene clusters have occurred in response to selection pressures exerted by the host.

In addition to *pap*, the F165-positive strains contained multiple virulence-associated genes characteristic of ExPEC strains. These included *sfa/focDE*, *afaE-8*, *F17c-A*, *clpG*, *bmaE*, *iucD*, *fyuA*, *traT*, *iss*, *ompT*, and *kpsMT* III. Interestingly, no toxin genes commonly associated with ExPEC strains were present in these strains. The secreted proteases are virulence factors in many bacterial and nonbacterial pathogens. In our study, the EspP protease was found in three F165-positive strains. EspP is associated with enterohemorrhagic *E. coli*, induces cytopathic effects, has been shown to cleave coagulation factor V, and may thus promote intestinal hemorrhage (9). This is the first report on the presence of the *espP* gene in ExPEC strains. The absence of many genes that characterize ExPEC strains such as strains J96, 536, and CFT073 may indicate that in F165-positive strains these PAIs could potentially acquire additional virulence genes over time or that the virulence genes within these putative PAIs were deleted. Furthermore, these three strains are isolates from human infections, as opposed to animal infections, and are associated with different pathologies (e.g., strain J96 was from a patient with pyelonephritis). This could reflect a difference in selective pressure that results in this ExPEC strain with specific characteristics.

The majority of PAIs detected in enterobacteria are specific for particular species or even pathotypes. The HPI, first described in pathogenic *Yersinia* (10), has been detected in many enterobacterial species and pathotypes, including both enteroaggregative *E. coli* and ExPEC (54). In addition, more than 30% of *E. coli* isolates from the normal intestinal microflora also carry this island (54). The functional core of the HPI in *Yersinia* consists of 12 genes (*irp1* to *irp9*, *ybtA*, *fyuA*, and *intB*). The mobility of the HPI elements may be associated with an intact integrase gene located at the left junction of the HPI.

TABLE 3. Pathogenicities of F165-positive strains

Group and strain	Source	Serogroup	No. of piglets inoculated	No. of piglets manifesting:		
				Mortality	Macro lesions[a]	Time of death after inoculation (h)
Group 1						
5131	Porcine intestine	O115:K"V165"	2	2	2	36–48
4787	Porcine intestine	O115:K"V165"	4	4	4	36–48
6389	Porcine intestine	O115:K"V165"	2	2	2	36–48
1776	Porcine intestine	O115:K"V165"	2	2	2	36–48
393	Porcine intestine	O115:K"V165"	2	2	2	40–48
Group 2						
2325	Bovine extraintestine	O11:KF12	2	2	2	48
3292	Bovine intestine	O15,O35,O11	NT[b]	NT	NT	NT
7867	Bovine intestine	O9:K28	2	2	2	48
3863	Bovine intestine	O9:K28	NT	NT	NT	NT
1401	Bovine intestine	O9:K28	2	2	1	20–30
3984	Porcine intestine	O101:K32	2	0	0	0
215	Bovine intestine	O?	NT	NT	NT	NT
Group 3						
2313	Bovine extraintestine	O15:KRVC383	2	0	1	0
2878	Bovine intestine	O15:KRVC383	2	0	0	0
1195	Porcine intestine	O9:K28	2	1	1	20
1616	Bovine intestine	O9:K28	NT	NT	NT	NT
3373	Porcine intestine	O9:K28	NT	NT	NT	NT
3719	Porcine intestine	O9:K28	NT	NT	NT	NT

[a] Macro lesions of polyserositis.
[b] NT, not tested.

The *fyuA*, *irp1*, and *irp2* genes were detected in all of the HPI-positive F165-positive strains. However, the yersiniabactin receptor FyuA was expressed in only seven of eight strains. The HPIs of F165-positive strains have features in common with the HPI elements of other enterobacteria, including pathogenic yersiniae. We showed that in six F165-positive *E. coli* strains, the HPI is inserted at the *asnT* tRNA gene. The *asnT* locus in *Yersinia* is linked to a gene whose sequence is highly homologous with that of a phage-derived integrase determinant termed *intB*. Furthermore, a copy of the IS*100* insertion sequence present in the *Yersinia pestis* HPI (at the 5′ end) is also present in the HPIs of F165-positive *E. coli* strains. The HPI elements code for a particular iron uptake system, termed yersiniabactin. The question arises as to whether the HPI elements in *E. coli* indeed represent PAIs and whether they contribute to the survival of the strains in certain ecological niches. Most strains of the family *Enterobacteriaceae* carry genes that encode high-affinity iron uptake systems, such as the enterobactin and aerobactin systems. Thus, the reason for a third iron uptake system in *E. coli*, like that encoded by the HPI, remains to be clarified. It is conceivable that the bacteria use different iron uptake systems, depending on the environment or the stage of infection. However, siderophores not only are involved in providing the microorganisms with iron but also can affect the cellular immune system (e.g., by suppression of T-cell proliferation) (54). This dual role of siderophores may contribute to the pathogenicities of members of the family *Enterobacteriaceae* and as a cause of extraintestinal infections. All HPI-positive F165 *E. coli* strains tested were pathogenic in piglets. Nevertheless, the HPI is not essential for some F165-positive strains, as shown by its absence from group 1 strains, which were pathogenic.

Four main phylogenetic groups (groups A, B1, B2, and D) were described by Herzer et al. (28) on the basis of examination of the 72 strains in the ECOR collection (46) by comparison of several genetic markers by multilocus enzyme electrophoresis. It may be relevant to these observations that isolates belonging to ECOR subgroup A have a significantly smaller genome than those belonging to either subgroup B2 or subgroup D, which have the largest genomes among natural isolates of *E. coli* (8). The *E. coli* genome varies from 4.5 to 5.5 Mb (49). For example, *E. coli* MG1655 has a genome of 4.63 Mb, and *E. coli* J96, which belongs to phylogenetic group B2, has a genome of 5.12 Mb (49). We determined the genome size of strain 4787, which belongs to phylogenetic group B1, by PFGE using three different restriction enzymes (*Sfi*I, *Not*I, and *Xba*I) (data not shown). The genome of strain 4787 is estimated to be about 4.9 Mb, which is larger than that of *E. coli* K-12 but smaller than that of pathogenic strain J96.

Although F165-positive strains have been isolated from clinical cases and most of them are pathogenic in experimental infections, they fall into groups A and B1 of the phylogenetic scheme proposed by Herzer et al. (28). In a study by Picard et al. (48), commensal strains belonging to phylogenetic groups A and B1 were shown to be devoid of virulence determinants and did not kill mice. Some strains of phylogenetic groups A, B1, and D were able to kill the mice and possessed virulence determinants. Strains of the B2 phylogenetic group are highly virulent and possess the highest level of virulence determinants. Certain F165-positive strains possess virulence attributes such as P-like fimbriae, HPIs, O serotypes, and phylogenetic groups that can be associated with pathogenicity, as tested in our model. F165-positive strains in phylogenetic group A possessed more virulence factors than those in phy-

logenetic group B1, but the strains in group B1 were more virulent in piglets than the strains in group A. It is not known why strains in our group 1 (which belong to phylogenetic group B1) are more virulent in our experimental model, despite the presence of fewer virulence markers than the numbers of virulence markers in strains of the two other groups. It is possible that some virulence genes have not yet been discovered or that these strains may contain a new PAI(s), or their virulence could be related to the presence of "black holes," genomic deletions that may enhance pathogenicity (44). The roles of the K"V165" O-antigen capsule and F165$_1$ fimbriae in bacterial pathogenicity were examined by comparing the pathogenicities of mutants in experimental infections. The F165$_1$ fimbriae and the K"V165" O-antigen capsule were strongly associated with bacterial survival in the extraintestinal organs and in the bloodstreams of infected piglets and with virulence. Fimbrial antigen F165$_1$ promotes adherence of bacteria to porcine polymorphonuclear leukocytes in vitro but enhances resistance to phagocytosis. O-antigen capsule K"V165" is required for resistance to the bacterial effects of serum and for resistance to phagocytosis by porcine polymorphonuclear leukocytes in vitro (20). It is also possible that certain factors, such as O type (e.g., O115, O78) and K serotype (K1), are important in key steps for survival in the blood and resistance to bacterial killing by the host, i.e., bacterial killing in the presence of complement and phagocytes in extraintestinal tissues and fluids. A recent study suggests that extracellular polysaccharides as well as type 1 fimbrae are preeminent virulence determinants in uropathogenic *E. coli* strains in the murine urinary tract (2).

Thus, F165-positive strains that possess such key pathogenic traits but that lack certain virulence markers found in ExPEC strains would be relatively more pathogenic than commensal organisms but less pathogenic or pathogenic in fewer situations than the strains in phylogenetic group B2. Nevertheless, our study has shown that strains belonging to phylogenetic groups B1 and A (such as F165-positive strains) are not merely commensal but might be considered opportunistic pathogens. Comparison of the presence of virulence factors (Table 2), the ability of the strains to cause disease (pathogenicity) (Table 3), and the phylogenetic group suggest that the pathogenic origin of the strains in group 1 could be related to other virulence factors which are unidentified and/or could be related to host-dependent factors.

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