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Sequence of Conjugative Plasmid pIP1206 Mediating Resistance to Aminoglycosides by 16S rRNA Methylation and to Hydrophilic Fluoroquinolones by Efflux▽

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Self-transferable IncFI plasmid pIP1206, isolated from an *Escherichia coli* clinical isolate, carries two new resistance determinants: *qepA*, which confers resistance to hydrophilic fluoroquinolones by efflux, and *rmtB*, which specifies a 16S rRNA methylase conferring high-level aminoglycoside resistance. Analysis of the 168,113-bp sequence (51% G+C) revealed that pIP1206 was composed of several subregions separated by copies of insertion sequences. Of 151 open reading frames, 56 (37%) were also present in pRSB107, isolated from a bacterium in a sewage treatment plant. pIP1206 contained four replication regions (RepFIA, RepFIB, and two partial RepFII regions) and a transfer region 91% identical with that of pAPEC-O1-ColBM, a plasmid isolated from an avian pathogenic *E. coli*. A putative *oriT* region was found upstream from the transfer region. The antibiotic resistance genes *tet*(A), *catA1*, bla_{TEM-1}, *rmtB*, and *qepA* were clustered in a 33.5-kb fragment delineated by two IS*26* elements that also carried a class 1 integron, including the *sulI*, *qacEΔ1*, *aad4*, and *dfrA17* genes and Tn*10*, Tn*21*, and Tn*3*-like transposons. The plasmid also possessed a raffinose operon, an arginine deiminase pathway, a putative iron acquisition gene cluster, an *S*-methylmethionine metabolism operon, two virulence-associated genes, and a type I DNA restriction-modification (R-M) system. Three toxin/antitoxin systems and the R-M system ensured stabilization of the plasmid in the host bacteria. These data suggest that the mosaic structure of pIP1206 could have resulted from recombination between pRSB107 and a pAPEC-O1-ColBM-like plasmid, combined with structural rearrangements associated with acquisition of additional DNA by recombination and of mobile genetic elements by transposition.

Plasmids are widespread in bacteria, where they represent an important part of the genome. Among these, conjugative plasmids are essential contributors to horizontal transfer of genetic information and to bacterial genome evolution. In addition, exchange of genetic material is enhanced by mobile genetic elements, such as transposons and insertion sequences (IS). Plasmids have been classified into incompatibility (Inc) groups on the basis of the impossibility for two plasmids to coexist stably within the same host (11, 14). Plasmids of the IncF group are considered of narrow host range. F-like plasmids are often (i) large molecules, from 40 to 200 kb that can harbor two or three sites for DNA replication initiation, (ii) conjugative, and (iii) composed of genes implicated in antibiotic resistance, virulence, and metabolic biochemical pathways. F-like plasmids are common in nature and have been found in 15% of an *Escherichia coli* collection containing 72 strains (10). IncFI self-transferable plasmid pIP1206 was detected in 2006 in *E. coli* 1540 during the screening of high-level aminoglycoside-resistant enterobacteria (8). pIP1206 contains the *catI*, *tet*(A), *dfrA17*, *sulI*, *qacEΔ1*, *aadA4*, and bla_{TEM-1} resistance genes that confer, respectively, resistance to chloramphenicol, tetracycline, trimethoprim, sulfonamides, quaternary ammonium compounds, streptomycin/spectinomycin, and β-lactams. Furthermore, pIP1206 carries genes responsible for two antibiotic resistance mechanisms recently detected in human pathogens: *rmtB*, which confers high-level resistance to 4,6-disubstituted deoxystreptamines by methylation at N-7 of residue G1405 of 16S rRNA (43), and *qepA*, which is responsible for diminished susceptibility to hydrophilic fluoroquinolones by synthesis of an efflux pump belonging to the major facilitator superfamily (MFS) (43).

Association of these two new genes with other antibiotic resistance determinants on the same plasmid is of concern, in particular because of the risk of dissemination due to coselection by various drugs. The aim of this work was to determine the genomic structure of pIP1206. This large plasmid presents similarities to pRSB107, originating from a bacterium in a sewage treatment plant (47), and pAPEC-O1-ColBM (24), isolated from an avian pathogenic *E. coli*, and it appears to be composed of DNA that has been acquired from various sources, probably following homologous recombination and transposition events.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* strain 1540 was isolated from a patient in a Belgian hospital during the screening for aminoglycoside resistance

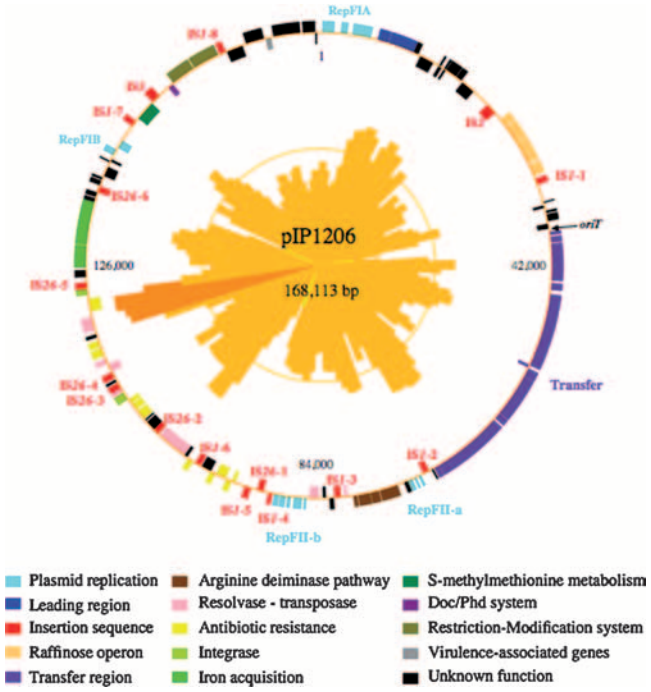

FIG. 1. Graphical map of pIP1206. The color code for the various gene functions is indicated below the map. The origin of transfer (*oriT*) is marked by a black arrow. The G+C plot is indicated on the inner circle (mean, 51%). G+C contents that were >2 standard deviations above and below the mean are indicated in dark orange and in yellow, respectively.

of clinical isolates of *Enterobacteriaceae* (8). *E. coli* C600Rif, a rifampin-resistant derivative of C600, *Acinetobacter* sp. strain R1 (20), and *Pseudomonas aeruginosa* PAO38 (21) were used as recipients in conjugation experiments. The strains were grown in brain heart infusion broth or on agar at 37°C.

Filter mating. Conjugation was performed by the solid mating-out assay with selection on rifampin (250 μg/ml) and kanamycin (40 μg/ml) for *E. coli* C600Rif and *Acinetobacter* sp. strain R1 or on rifampin (32 μg/ml) and kanamycin (250 μg/ml) for *P. aeruginosa* PAO38.

DNA sequencing. Plasmid pIP1206 DNA was prepared by the alkaline lysis procedure (7). The sequence was determined by the shotgun cloning method (MWG Biotech, Champlan, France). The pGEM-Teasy vector was electroporated into *E. coli* DH10B, which is T1 phage resistant. Primers used for DNA sequencing were easyR, 5′-CAGGCGGCCGCGAATTCAC-3′, and easyF, 5′-TGGCGGCCGCGGGAATCCG-3′.

Sequence analysis. Contig assembly and annotation were performed using the Contigs-Assembly and Annotation Tool-Box (CAAT-Box) software (16) developed at the Genopole of Institut Pasteur for the computational analysis of sequences obtained by the shotgun method, such as prediction of links between contigs as well as the annotation of the sequence. CAAT-Box was also used to create the EMBL file.

Nucleotide sequence accession number. The sequence of pIP1206 was submitted to the EMBL database and assigned accession number AM886293.

RESULTS AND DISCUSSION

General structure of pIP1206. The sequence of pIP1206, isolated from *E. coli* 1540, was determined by a shotgun approach. The plasmid was found to be a circular molecule of 168,113 bp (Fig. 1) with a G+C content of 51%, ranging from 30% to 72%. Annotation of the data using the CAAT-Box tool (16) resulted in the detection of 176 coding sequences. Among these, 107 were assigned to six functions: replication, transfer, antibiotic resistance, biochemical pathways, restriction-modification (R-M), and virulence, whereas 44 encoded hypothetical proteins (Fig. 1 and Table 1). Of these 151 open reading frames (ORFs), 56 (37%) were also present in pRSB107, which was isolated from a bacterium in a sewage treatment plant (47), 44 (29%) in pAPEC-O1-ColBM (24), which was isolated from an avian pathogenic *E. coli*, 11 (7%) in both plasmids, and 40 (27%) from various sources. Furthermore, 25 coding sequences as part of 16 ISs, in particular IS*26* and IS*1*, were found throughout the plasmid. It is noteworthy that (i) some sets of genes in pRSB107 are not present in pIP1206 and (ii) an inversion occurred that resulted in the presence of a Tn*21*-like sequence in both pRSB107 and pIP1206 but in the opposite orientation. Furthermore, it is likely that the IS copies also are responsible for inversion events. It thus appears that pIP1206 could result from acquisition by a pRSB107-like plasmid, which differs from prototype pRSB107 following deletions and inversions of several determinants by transpositions mediated by insertion sequences or transposons and of part of pAPEC-O1-ColBM-like plasmid, such as the entire transfer region, by homologous recombination.

Replication regions. Plasmid pIP1206 contained four replication regions, RepFIA, RepFIB (Fig. 1), RepFII-a, and RepFII-b (Fig. 1 and 2). RepFIA, a 6,498-bp fragment (ORFs 1 to 6; G+C content, 47%) contained the *oriV-1* origin of replication and the genes necessary for maintenance of the plasmid, such as DNA replication (*repE*), partition (the *ccdA*/*ccdB* and *sopABC* operons), multimer resolution (*resD*), and incompatibility (*incC*), and was 99% identical to the RepFIA primary replication region of pRSB107 (47).

The *ccdA-ccdB* antitoxin-toxin operon (22) enforces stabilization of the plasmid. The CcdB toxin acts on the A subunit of the gyrase, an essential topoisomerase involved in DNA replication, recombination, and transcription, by catalyzing ATP-dependent negative supercoiling of DNA (35). The CcdB-gyrase complex is responsible for the relaxation of supercoiled DNA. The toxic effect of CcdB is antagonized by CcdA, but the half-life of the CcdA antitoxin is shorter than that of CcdB.

The *resD* gene encodes a recombinase that resolves multimers to monomers. Partition of the plasmid is ensured by the *sopABC* cluster, which is responsible for adequate subcellular positioning of plasmid DNA molecules and is thus necessary for stable maintenance of low-copy-number F-plasmids (40, 41). *sopABC* of pIP1206 was identical to that in pRSB107.

RepE binds specifically to four *incB* iterons, which are 19-bp directly repeated sequences in *oriV-2*, and causes bending of this region, which is a critical first step in replication of F-plasmids (42, 57). The *incC* region is implicated in incompatibility with plasmids of the same group and in the control of the plasmid copy number (49, 51).

The second replication region, RepFIB (ORFs 154 and 155; G+C content, 52%) is composed of a *repA* replication and an *int* site-specific integrase gene that share more than 99% identity with the RepFIB replicon from several IncF plasmids, including pRSB107 and pAPEC-O1-ColBM. Two genes (ORFs 148 and 149) encoding proteins putatively involved in the regulation of plasmid copy number and in plasmid stabilization were found upstream from the *int* gene.

Two RepFII replicons (FII-a, ORFs 76 to 79 and G+C content of 51%; FII-b, ORFs 92 to 98 and G+C content of 52%) were also present in pIP1206 (Fig. 2). FII-a was composed of genes

TABLE 1. ORFs identified in pIP1206

ORF	Gene	G+C (%)	Function	Identity (%)	Pfam[a]	Accession no.	Organism (plasmid)
			oriV-1 region	86		AJ851089	Uncult.[b] (pRSB107)
1	*ccdA*	51	Antitoxin	100	07362	AJ851089	Uncult. (pRSB107)
2	*ccdB*	50	Toxin	100	01845	AJ851089	Uncult. (pRSB107)
3	*resD*	57	Resolvase	100	00589	AJ851089	Uncult. (pRSB107)
4	*repE*	47	Replication protein (RepE)	100	01051	AJ851089	Uncult. (pRSB107)
5	*sopA*	47	Partitioning protein	100	01656	AJ851089	Uncult. (pRSB107)
6	*sopB*	45	Partitioning protein	100	02195	AJ851089	Uncult. (pRSB107)
7	*orf7*	55	Hypothetical protein	100	06924	AJ851089	Uncult. (pRSB107)
8	*orf8*	63	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
9	*orf9*	56	DNA methylase	100	01555	AJ851089	Uncult. (pRSB107)
10	*orf10*	53	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
11	*orf11*	57	Hypothetical protein	100	07128	AJ851089	Uncult. (pRSB107)
12	*orf12*	60	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
13	*orf13*	63	Hypothetical protein	98	07339	NC_002134	*E. coli* (pR100)
14	*klcA*	55	Hypothetical antirestriction	100	03230	AJ851089	Uncult. (pRSB107)
15	*orf15*	56	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
16	*orf16*	51	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
17	*orf17*	49	RNA-directed DNA polymerase	62	08388	NC_008322	*Shewanella* sp.
18	*ydaA*	68	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
19	*orf19*	61	Hypothetical protein	97		NC_006671	*E. coli* (pAPEC-O2-R)
20	*orf20*	58	Hypothetical protein	100		NC_002134	*E. coli* (pR100)
21	*orf21*	61	Hypothetical protein	98		NZ_AAJX01000096	*E. coli*
22	*ydbA*	56	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
23	*orf23*	60	Hypothetical DNA methylase	96		NC_006671	*E. coli* (pAPEC-O2-R)
24	*orf24*	61	Hypothetical protein	100		NC_006671	*E. coli* (pAPEC-O2-R)
25	*orf25*	49	RNA-directed DNA polymerization	62	08388	NC_008322	*Shewanella* sp.
26	IS*2*-*orf2*	54	Transposase	98	00665	AC_000091	*E. coli*
27	IS*2*-*orf1*	54	Transposase	99	01527	AC_000091	*E. coli*
28	*rafR*	48	Transcriptional regulator	89	00356	M29849	*E. coli* (pRSD2)
29	*rafA*	51	α-Galactosidase	97	02065	M27273	*E. coli* (pRSD2)
30	*rafB*	46	Raffinose permease	97	01306	M27273	*E. coli* (pRSD2)
31	*rafD*	51	Raffinose invertase	96	08244	M27273	*E. coli* (pRSD2)
32	*rafY*	39	Putative glycoporine	88		U82290	*E. coli* (pRSD2)
33	IS*1*-*insA*	52	Transposase	97	03811	NC_006816	*Salmonella enterica* serovar Typhimurium (pU302L)
34	IS*1*-*insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
35	*orf35*	47	Stable inheritance protein	30	07885	NC_002483	*E. coli* (plasmid F)
36	*yubN*	61	Hypothetical protein	90		NC_002483	*E. coli* (plasmid F)
37	*yubO*	52	Hypothetical protein	95		NC_002483	*E. coli* (plasmid F)
38	*yubP*	44	Hypothetical protein	99	06067	DQ381420	*E. coli* (pAPEC-O1-ColBM)
39	*orf39*	43	Transglycosylation	95	01464	DQ381420	*E. coli* (pAPEC-O1-ColBM)
40	*traM*	38	Mating signal	99	05261	NC_009602	*E. coli* (pSFO157)
41	*traJ*	33	Regulation	96		M62986	*E. coli* (IncFI p307)
42	*traY*	38	*oriT* nicking	95		M62986	*E. coli* (IncFI p307)
43	*traA*	52	F pilin subunit	98	05513	DQ381420	*E. coli* (pAPEC-O1-ColBM)
44	*traL*	47	F pilus assembly	100	07178	DQ381420	*E. coli* (pAPEC-O1-ColBM)
45	*traE*	47	F pilus assembly	99	05309	DQ381420	*E. coli* (pAPEC-O1-ColBM)
46	*traK*	57	F pilus assembly	98	06586	DQ381420	*E. coli* (pAPEC-O1-ColBM)
47	*traB*	57	F pilus assembly	99	06447	DQ381420	*E. coli* (pAPEC-O1-ColBM)
48	*traP*	49	Conjugal transfer protein	99	07296	DQ381420	*E. coli* (pAPEC-O1-ColBM)
49	*trbG*	42		100		DQ381420	*E. coli* (pAPEC-O1-ColBM)
50	*traV*	52	F pilus assembly	97		DQ381420	*E. coli* (pAPEC-O1-ColBM)
51	*traC*	53	F pilus assembly	98		DQ381420	*E. coli* (pAPEC-O1-ColBM)
52	*trbI*	58		98		DQ381420	*E. coli* (pAPEC-O1-ColBM)
53	*traW*	60	F pilus assembly	98		DQ381420	*E. coli* (pAPEC-O1-ColBM)
54	*traU*	55	F pilus assembly	97	06834	DQ381420	*E. coli* (pAPEC-O1-ColBM)
55	*trbC*	55	F pilus assembly	98		DQ381420	*E. coli* (pAPEC-O1-ColBM)
56	*traN*	52	Aggregate stability	99	06986	DQ381420	*E. coli* (pAPEC-O1-ColBM)
57	*trbE*	41		100		DQ381420	*E. coli* (pAPEC-O1-ColBM)
58	*traF*	50	F pilus assembly	99		DQ381420	*E. coli* (pAPEC-O1-ColBM)
59	*trbA*	38	Conjugal transfer protein	95		NC_007941	*E. coli* (pUTI89)
60	*artA*	33		96		NC_007941	*E. coli* (pUTI89)
61	*traQ*	54	F pilin synthesis	95		NC_007941	*E. coli* (pUTI89)
62	*trbB*	57		96		DQ381420	*E. coli* (pAPEC-O1-ColBM)
63	*trbJ*	47	Conjugal transfer protein	69		DQ381420	*E. coli* (pAPEC-O1-ColBM)
64	*trbF*	38		60		NC_007941	*E. coli* (pUTI89)

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TABLE 1—*Continued*

ORF	Gene	G+C (%)	Function	Identity (%)	Pfam[a]	Accession no.	Organism (plasmid)
65	*traH*	51	F pilus assembly	99	06122	NC_006671	*E. coli* (pAPEC-O2-R)
66	*traG*	50	F pilus assembly	92	07916	DQ381420	*E. coli* (pAPEC-O1-ColBM)
67	*traS*	30	Surface exclusion	54		DQ381420	*E. coli* (pAPEC-O1-ColBM)
68	*traT*	50	Surface exclusion	99	05818	DQ381420	*E. coli* (pAPEC-O1-ColBM)
69	*traD*	52	DNA transport	96	02534	DQ381420	*E. coli* (pAPEC-O1-ColBM)
70	*traI*	59	DNA helicase	96	07057	DQ381420	*E. coli* (pAPEC-O1-ColBM)
71	*traX*	57	F pilin acetylation	97	05857	AJ851089	Uncult. bact. (pRSB107)
72	*finO*	55	Fertility inhibition protein	97	05286	DQ381420	*E. coli* (pAPEC-O1-ColBM)
73	*orf73*	61	Hypothetical protein	92		DQ381420	*E. coli* (pAPEC-O1-ColBM)
74	IS*1-insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
75	IS*1-insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
76	*srnB*	49	Postsegregation killing	96		DQ381420	*E. coli* (pAPEC-O1-ColBM)
77	*repA2*	46	Regulation of RepA1 expression	84		DQ381420	*E. coli* (pAPEC-O1-ColBM)
78	*repA6*	49	Regulation of RepA1 expression	83		AJ851089	Uncult. (pRSB107)
79	Δ*repA1*	56	Replication initiation (truncated)	50	02387	AJ851089	Uncult. (pRSB107)
80	*orf80*	50	Hypothetical protein				
81	*arcA*	53	Arginine deiminase	95	02274	CP000653	*Enterobacter* sp. strain 638
82	*arcC*	57	Carbamate kinase	88	00696	CP000653	*Enterobacter* sp. strain 638
83	*arcB*	53	Ornithine carbamoyl transferase	91	00185	CP000653	*Enterobacter* sp. strain 638
84	*arcD*	54	C-4 dicarboxylate anaerobic carrier	91	03606	CP000653	*Enterobacter* sp. strain 638
85	*argR*	48	Arginine repressor	84	01316	CP000653	*Enterobacter* sp. strain 638
86	*orf86*	52	Putative transposase	98	05717	NC_002655	*E. coli*
87	IS*1-insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
88	IS*1-insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
89	*orf89*	52	Truncated hypothetical protein	84		AY545598	*E. coli* (pAPEC-O2-ColV)
90	*orf90*	43	Hypothetical protein	69		NC_008253	*E. coli*
91	*orf91*	49	Transposase	95	02371	AF389912	*S. marcescens*
92	*repA2*	47	Regulation of RepA1 expression	97		NC_007635	*E. coli* (pCoo)
93	*repA6*	49	Regulation of RepA1 expression	83		AJ851089	Uncult. (pRSB107)
94	*repA1*	56	Replication initiation	96	02387	AJ851089	Uncult. (pRSB107)
95	*repA4*	58	Replication protein	99		V00351	*E. coli* (pRSC13)
96	*tir*	52	Transfer inhibition	100	02517	AJ851089	Uncult. (pRSB107)
97	*pemI*	53	Plasmid stable inheritance	100	04014	AJ851089	Uncult. (pRSB107)
98	*pemK*	55	Plasmid stable inheritance	100	02452	AJ851089	Uncult. (pRSB107)
99	IS*1-insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
100	ΔIS*1-insB*	52	Transposase (truncated)	64	03400	NC_006816	Serovar Typhimurium (pU302L)
101	IS*26-tnpA*	52	Transposase	100	00665	NC_006856	*S. enterica*
102	IS*1-insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
103	IS*1-insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
104	*tetD*	35	Tetracycline resistance	100	00165	AJ851089	Uncult. (pRSB107)
105	*tetC*	32	Tetracycline transcriptional regulator	100	00440	AJ851089	Uncult. (pRSB107)
106	*tetA*	43	MFS efflux pump	99	07690	AJ851089	Uncult. (pRSB107)
107	*tetR*	40	Tetracycline repressor protein	100	02909	AJ851089	Uncult. (pRSB107)
108	*jemC*	41	Transcriptional regulator	100	01022	AJ851089	Uncult. (pRSB107)
109	*orf109*	37	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
110	IS*1-insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
111	IS*1-insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
112	*catA*	44	Chloramphenicol resistance	100	00302	AP000342	*E. coli* (pR100)
113	*ybjA*	48	Acetyltransferase	100	00583	AP000342	*E. coli* (pR100)
114	*tnpA*-Tn*21*	63	Transposase	100	01526	AP000342	*E. coli* (pR100)
115	*tnpR*-Tn*21*	57	Resolvase	100	02796	AP000342	*E. coli* (pR100)
116	IS*26-tnpA*	52	Transposase	100	00665	NC_006856	*S. enterica*
117	Δ*chrA*	58	Chromate ion transport (truncated)	100	02417	AJ698325	Uncult. (pRSB101)
118	*orf118*	62	Hypothetical protein	100		DQ390454	*E. coli* (pLEW517)
119	*sulI*	62	Sulfonamide resistance	99	00809	AP000342	*E. coli* (pR100)
120	*qacE*Δ*1*	50	Quaternary ammonium compound resistance	100	00893	AP000342	*E. coli* (pR100)
121	*aadA4*	58	Streptomycin-spectinomycin resistance	100	01909	AY214164	*E. coli* (pAPEC-O2-R)
122	*dfrA17*	36	Trimethoprim resistance	100	00186	AY828551	*E. coli*
123	*int*	61	Integrase	100	02899	AP000342	*E. coli* (pR100)
124	Δ*tnpM*	63	Transposase (truncated)	81	00563	AJ851089	Uncult. (pRSB107)
125	IS*26-tnpA*	52	Transposase	100	00665	NC_006856	*S. enterica*
126	*orf126*	55	Hypothetical protein	100	03551	AJ698325	Uncult. (pRSB101)
127	IS*26-tnpA*	52	Transposase	100	00665	NC_006856	*S. enterica*

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TABLE 1—*Continued*

ORF	Gene	G+C (%)	Function	Identity (%)	Pfam[a]	Accession no.	Organism (plasmid)
128	Δ*tnpA*-Tn*3*	51	Transposase (truncated)	99	01526	CP000057	*H. influenzae*
129	*tnpR*-Tn*3*	51	Resolvase	99	02796	CP000057	*H. influenzae*
130	bla_{TEM-1}	49	β-Lactam resistance	100	00144	AB070224	*S. marcescens*
131	*rmtB*	56	Aminoglycoside resistance	100	07091	AB103506	*S. marcescens*
132	*orf132*	59	Hypothetical protein				
133	*orf133*	69	Transposase	99	04986	CT025832	*A. baumannii*
134	*qepA*	72	Fluoroquinolone resistance	56	07690	CP000316	*Polaromonas* sp.
135	Δ*int*	61	Integrase (truncated)	100	02899	DQ390454	*E. coli* (pLEW517)
136	IS*26*-*tnpA*	52	Transposase	100	00665	NC_006856	*S. enterica*
137	*nqrc*	47	NADH-ubiquinone oxidoreductase	100	04205	AJ851089	Uncult. (pRSB107)
138	*orf138*	55	Hypothetical protein	99	03239	AJ851089	Uncult. (pRSB107)
139	*orf139*	53	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
140	*orf140*	57	Hypothetical protein	100	04945	AJ851089	Uncult. (pRSB107)
141	*orf141*	59	Hypothetical protein	100	02687	AJ851089	Uncult. (pRSB107)
142	*orf142*	58	Hypothetical protein	100	02687	AJ851089	Uncult. (pRSB107)
143	*orf143*	57	ABC transporter ATP binding protein	100	00005	AJ851089	Uncult. (pRSB107)
144	*orf144*	58	Hypothetical protein	100	08534	AJ851089	Uncult. (pRSB107)
145	*orf145*	49	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
146	*orf146*	57	Glucose-1-phosphatase	100	00328	AJ851089	Uncult. (pRSB107)
147	IS*26*-*tnpA*	52	Transposase	100	00665	CT025832	*A. baumannii*
148	*orf148*	54	Hypothetical protein	100	01402	AJ851089	Uncult. (pRSB107)
149	*orf149*	48	Plasmid stabilization system	100	05016	AJ851089	Uncult. (pRSB107)
150	*orf150*	38	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
151	*orf151*	33	Hypothetical protein	100		AJ851089	Uncult. (pRSB107)
152	*orf152*	49	Hypothetical protein	100		NC_006816	Serovar Typhimurium (pU302L)
153	*orf153*	45	Hypothetical protein				
154	*int*	57	Integrase	99	00589	AJ851089	Uncult. bact. (pRSB107)
155	*repA*	51	Replication	99	01051	AJ851089	Uncult. bact. (pRSB107)
156	IS*1*-*insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
157	IS*1*-*insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
158	*mmuM*	60	Homocysteine *S*-methyltransferase	100	02574	U70214	*E. coli*
159	*mmuP*	54	*S*-methylmethionine permease	99	00324	U70214	*E. coli*
160	IS*5*-*tnpA*	55	Transposase	99	01609	AJ001620	*E. coli*
161	*doc*	52	Death on curing protein	99	05012	AF234173	Bacteriophage P1
162	*phd*	51	Prevent host death protein	100	02604	AF234173	Bacteriophage P1
163	*hsdM*	45	Type I restriction enzyme, methylase	99	02384	X13145	*E. coli* (pR124/3)
164	*hsdS*	39	Type I restriction enzyme, specificity	57	01420	CP000447	*S. frigidimarina*
165	*hsdR*	43	Type I restriction enzyme, nuclease	97	04313	X13145	*E. coli* (pR124/3)
166	IS*1*-*insA*	52	Transposase	97	03811	NC_006816	Serovar Typhimurium (pU302L)
167	IS*1*-*insB*	52	Transposase	99	03400	NC_006816	Serovar Typhimurium (pU302L)
168	*orf168*	48	Hypothetical protein	23		AAMR01000022	*V. splendidus*
169	*orf169*	49	Hypothetical protein	41		AAMR01000022	*V. splendidus*
170	*orf170*	49	Hypothetical protein	100		DQ381420	*E. coli* (pAPEC-O1-ColBM)
171	*vagD*	59	Virulence-associated protein	99	01850	DQ381420	*E. coli* (pAPEC-O1-ColBM)
172	*vagC*	54	Virulence-associated protein	100	04014	DQ381420	*E. coli* (pAPEC-O1-ColBM)
173	*orf173*	44	Hypothetical protein	89		DQ449578	*Klebsiella pneumoniae* (pK245)
174	*orf174*	50	Hypothetical protein	100		DQ381420	*E. coli* (pAPEC-O1-ColBM)
175	*korC*	57	Hypothetical protein	100		DQ381420	*E. coli* (pAPEC-O1-ColBM)
176	*orf176*	45	Hypothetical protein	100		DQ381420	*E. coli* (pAPEC-O1-ColBM)

[a] Pfam is a large collection of multiple sequence alignments and hidden Markov models covering many common protein domains and families.
[b] Uncult., uncultured bacterium.

encoding SrnB, involved in postsegregational killing of plasmid-free cells, and RepA2 and RepA6, responsible for negative and positive regulation of RepA1 transcription, respectively. RepA2 shared 84% and 48% identity with RepA2 of pAPEC-O1-ColBM and pRSB107, respectively. Downstream from these genes, there was a 3′-end truncated *repA1* gene. FII-b, the second RepFII replicon, was composed of the *repA2*, *repA6*, *repA1*, and *repA4* genes. The $RepA2_{FII-b}$ protein was 48%, 52%, and 61% identical with $RepA2_{FII-a}$ of pIP1206, pAPEC-O1-ColBM, and pRSB107, respectively. $RepA6_{FII-b}$ was identical with $RepA6_{FII-a}$ of pIP1206 and had 83% identity with $RepA6_{pRSB107}$; there is no report of RepA6 in pAPEC-O1-ColBM. The *repA4* region shared 97% identity with $repA4_{pRSB107}$ and only 20% with that of $repA4_{pAPECO1-ColBM}$. However, a mutation in the 5′ portion of $repA4_{pRSB107}$ is responsible for a truncated RepA4 of only 4 amino acids. As in pRSB107, the *tir*, *pemI*, and *pemK* genes were present downstream from *repA4*. The *tir* gene encodes a putative protease, and *pemI*/*pemK* were similar to *chpAI*/*chpAK* (69% and

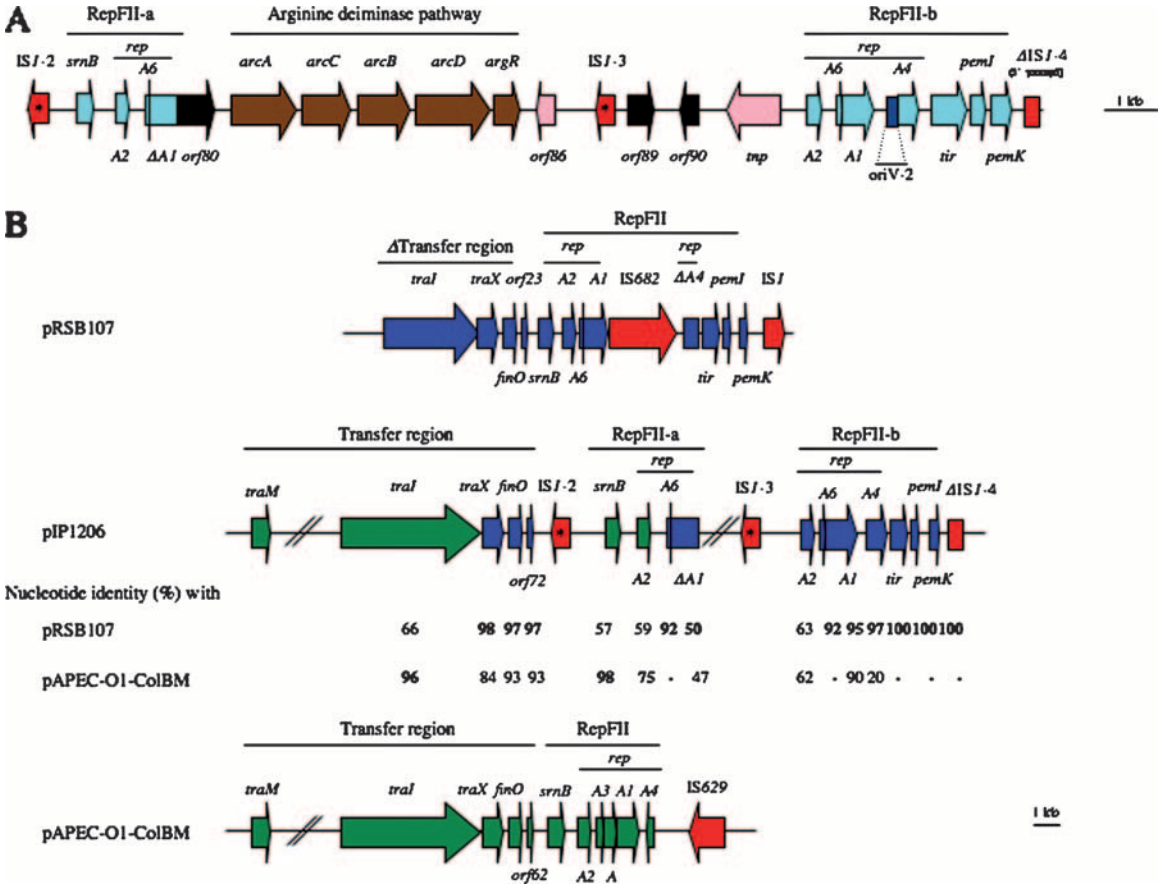

FIG. 2. (A) Schematic representation of the 19-kb fragment of pIP1206 containing the RepFII replicons and the genes for the arginine deiminase pathway. The RepFII genes are shown in blue, those of the arginine pathway in brown, IS elements in red, transposase in pink, and ORFs for hypothetical proteins in black. The OriV-2 replication origin is shown in dark blue. (B) Comparison of the RepFII regions of pRSB107, pIP106, and pAPEC-01-ColBM. The genes in pIP1206 exhibiting the highest level of identity with pRSB107 are indicated in blue, and those with identify with pAPEC-01-ColBM are in green. Insertion sequences are in red. Asterisks indicate a 4-bp deletion in *insB′* of IS*1* sequences IS*1-2* and IS*1-3*. Arrows represent coding sequences and indicate the direction of transcription. The Δ symbol denotes a deletion.

81% identity, respectively), which have properties of an addiction module, ChpAI, protecting the bacterium from the toxic effect of ChpAK (15, 38). As in pRSB107, an IS*1* copy was located downstream from FII-b (47). In summary, these data indicate that the two RepFII replicons have distinct origins and suggest that pIP1206 could have resulted from a double homologous recombination event between pRSB107 and a pAPEC-O1-ColBM-like plasmid.

IncF plasmids frequently possess more than one replication region, and it is thought that this could provide the plasmid with a broader range of hosts. However, we could not obtain, in repeated attempts, transfer of pIP1206 from *E. coli* Top10/pIP1206 to *Acinetobacter* sp. strain R1 and to *P. aeruginosa* PAO38 by filter mating. These results indicate that, despite the presence of four replication regions, pIP1206 is, as shown for other plasmids of the IncF group, of narrow host range.

The pIP1206 leading region is highly similar to that of pRSB107. Downstream from RepFIA, a 5,179-bp fragment (ORFs 7 to 16; G+C content, 58%) was identical to a portion in pRSB107. This region, highly similar to the F-plasmid leading region, is the first piece of DNA to enter the recipient cell during conjugative transfer (47). In pIP1206 and pRSB107, this region is composed of 10 genes encoding eight proteins with unknown functions, ORF9 for a DNA methylase, and ORF14 for a putative antirestriction protein (Fig. 1). The methylase is a member of the N^6-methyltransferase group that has been proposed to protect the transferred DNA from endonucleases in the recipient cell (37). Surprisingly, another gene for a methyltransferase (ORF23), partially present in pRSB107, was located downstream from the leading region. This methylase, which is very distantly related to the previous one (only 15% identity), was 97% identical to an N^6-methyltransferase encoded by pAPEC-O1-ColBM (accession number DQ381420). Of note, the *ydbA* gene, located just upstream from ORF23 in pIP1026, is also present in pRSB107 and pAPEC-O1-ColBM. Thus, these two ORFs could be implicated in a double homologous recombination event between pRSB107 and pAPEC-O1-ColBM.

Two copies (ORFs 17 and 25; 99.3% identity; 49% G+C content) of an additional gene for a putative reverse transcriptase were found in pIP1206 downstream from the plasmid

leading region. These proteins exhibited 62% identity with an RNA-directed DNA polymerase found in *Shewanella* sp. (accession number CP00034).

Transfer region. Upstream from RepFII-a, a 32,029-bp fragment (ORFs 40 to 72; G+C content, 52%) contained a complete transfer region (Fig. 1) with 91% identity to that of pAPEC-O1-ColBM. This region comprised 24 *tra* genes (*traM*, *traJ*, *traY*, *traA*, *traL*, *traE*, *traK*, *traB*, *traP*, *traV*, *traR*, *traC*, *traW*, *traU*, *traN*, *traF*, *traQ*, *traH*, *traG*, *traS*, *traT*, *traD*, *traI*, and *traX*), and 8 *trb* genes (*trbG*, *trbI*, *trbC*, *trbE*, *trbA*, *trbB*, *trbF*, and *trbJ*), *artA*, and *finO*. Twenty-three of the predicted proteins shared more than 95% identity with those encoded by pAPEC-O1-ColBM; the level of identity was lower for TraM (82%), TraJ (26%), TraY (25%), TrbA (58%), TrbF (69%), TrbJ (69%), TraG (92%), TraS (66%), TraX (88%), ArtA (74%), and FinO (94%). A frameshift mutation in the *trbJ* gene of pIP1206 resulted in a premature stop codon and thus to synthesis of a truncated protein. The function of the putative inner membrane protein TrbJ is unknown. However, mutations in *trbJ* do not significantly alter transfer efficiency (36). TraJ and TraY, implicated in the transcriptional regulation of *tra* gene expression (17), were highly similar to their counterparts in IncFI plasmid P307 (Table 1). TraD of pIP1206 contained a triplication of the 621-PQQ-623 motif compared to the sequence deduced from pAPEC-O1-ColBM. The number of repetitions of this motif in TraD seems to be polymorphic: 6 in pAPEC-O2-ColV (accession number NC_007675) and pAPEC-O2-R (accession number NC_006671), 7 in pSFO157 (accession number NC_009602), R1 (accession number AY684127), and pC15-1a (accession number NC_005327), 9 in R100 (accession number NC_002134), 10 in NR1 (accession number NC_009133), and 13 in pSS_046 (accession number NC_007385). Transfer by conjugation is initiated by site- and strand-specific nicking of plasmid DNA at the *oriT* transfer origin, which is located near the transfer region. Upstream from $traM_{pIP1206}$, a 432-bp fragment exhibited 61% identity with the *oriT* region of plasmid R100 (Fig. 3) (1). Eight recognition and binding sites, *sbi*, *ihfA*, *sbyA*, *sbmA*, *sbmB*, *ihfB*, *sbmC*, and *sbmD*, have been shown to be involved in DNA transfer (Fig. 3). In pIP1206, these eight sequences were present and shared 89%, 86%, 71%, 53%, 61%, 61%, 38%, and 47% identity, respectively, with their counterparts in R100. It has been suggested that another putative functional site, located between gene *X* and the *sbi* site in R100 (Fig. 3), is implicated in efficient DNA transfer. In pIP1206, a 99% identical fragment was found (Fig. 3). Furthermore, the *X* gene of R100 and *orf39* of pIP1206, which are located upstream from *traM*, exhibited 92% identity. These observations strongly suggest that pIP1206 possesses a functional *oriT*. Transfer of plasmid pIP1206 from *E. coli* Top10/pIP1206 to *E. coli* C600Rif was obtained at a frequency of 10^{-4} per donor. This result confirmed that, despite the presence of a mutated *trbJ* and of *trbF* and *traS* exhibiting unusual sequences, the transfer region of pIP1206 is functional.

Plasmid pRSB107 contains an incomplete transfer region composed of part of *traI*, *traX*, and *finO*. Deduced amino acid sequences revealed that TraI of pIP1206 shared a higher level of identity with TraI of pAPEC-O1-ColBM than that of pRSB107, whereas TraX and FinO presented a higher identity with the corresponding genes in pRSB107 (Fig. 2B). Thus, the *traI traX finO* region could be implicated in the homologous recombination that took place between pRSB107 and a pAPEC-O1-ColBM-like plasmid to generate pIP1206.

Raffinose operon and cluster for an arginine deiminase pathway. A raffinose operon (ORFs 28 to 32; G+C content, 47%) was detected in pIP1206 between the replication and the transfer regions (Fig. 1). It was composed of five genes encoding a repressor (RafR), an α-D-galactosidase (RafA), a permease (RafB), a sucrose hydrolase (RafD), and a porin (RafY) (2, 4, 52). These proteins were 89%, 97%, 97%, 96%, and 88% identical to those encoded by the raffinose operon in plasmid pRSD2, which allows growth of *E. coli* on raffinose (4). Between the *rafR* and *rafA* genes were two symmetrical 18-bp sequences form the *rafO* operator (5). The sequence of *rafO* from pIP1206 exhibited 90% identity with that from pRSD2.

An *arcACBD* gene cluster was located downstream from the transfer region (ORFs 81 to 85; G+C content, 53%) (Fig. 2A) encoding proteins involved in the arginine deiminase pathway, one of the four arginine catabolic pathways (for a review see reference 30). The pIP1206 gene set *arcA*, *arcB*, and *arcC* encoded, respectively, an arginine deiminase, an ornithine transcarbamylase, and a carbamate kinase. These proteins exhibited 95%, 91%, and 88% identity with their counterparts in *Enterobacter* sp. strain 638 (Table 1). The deduced amino acid sequence of the additional ORF84 (*arcD*) downstream from *arcB* for ornithine transcarbamylase shared, respectively, 92% and 82% identity with a protein previously described in GenBank as a C-4 dicarboxylate anaerobic carrier (CduC) from *Enterobacter* sp. strain 638 (accession number CP000653) and as an arginine-ornithine antiporter in *Aeromonas salmonicida* subsp. *salmonicida* (accession number CP000462). In these organisms, the protein is also specified by a gene downstream from *arcB*. The arginine biosynthetic pathway is regulated by a repressor (ArgR) (for a review, see reference 31). ArgR of pIP1206 had 84% identity with ArgR of *Enterobacter* sp. strain 638. *arc* operons have been detected in various species, but the gene order differs: *arcDABC* in *Pseudomonas aeruginosa* (9, 53), *arcABCRD* in *Enterococcus faecalis* (6), and *arcABDCR* in *Bacillus licheniformis* (33). In pIP1206, the gene organization (*arcACBDR*) was identical to that in *Enterobacter* sp. strain 638.

Mobile elements in pIP1206. Insertion sequences can be responsible for DNA rearrangements (deletions, duplications, or inversions) that are associated with transposition (34). Class I transposons consist of a DNA segment flanked by two copies of an IS, in direct or in opposite orientation.

Analysis of pIP1206 revealed the presence of 16 copies of IS elements: six IS*26*, four IS*1*, three mutated IS*1*, one IS*1* truncated at the 3′ end, one IS*2*, and one IS*5* (Fig. 1). The three mutated IS*1* elements had the same deletion of four bases (351-CAGT-354) in the *insA*-*insB* intergenic region, precisely within the *insB′* ORF, which is a 5′ extension of *insB*. It has been demonstrated that *insA* and *insB*, but not *insB′*, are essential for cointegrate formation. A mutation in the latter ORF is responsible for a reduction in cointegration frequency (23, 32). Thus, these three IS*1* copies are potentially functional. Three transposons, Tn*3*, Tn*10* and a Tn*21*-like transposon, were identified, but none of them was intact. Three genes (ORFs 86, 91, and 133) encoding putative transposases were also found throughout pIP1206.

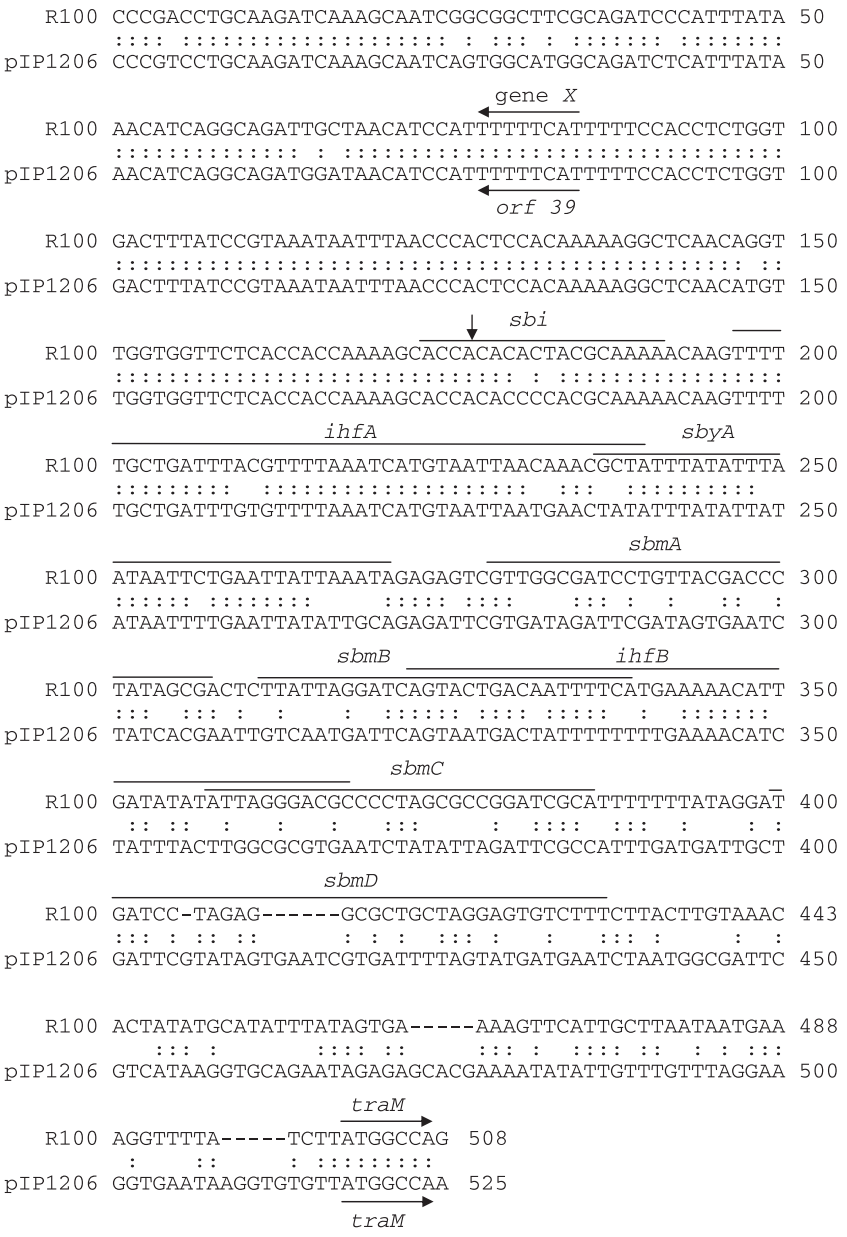

FIG. 3. Sequence comparison of the *oriT* regions of plasmids R100 and pIP1206. The TraI recognition site (*sbi*) and the TraY (*sbyA*), TraM (*sbmA*, *-B*, *-C*, and *-D*), and integration host factor (*ihfA* and *-B*) binding sites are indicated and are as defined previously (1). The vertical arrow indicates the *oriT* origin of DNA transfer.

The movement of transposons or insertion sequences is usually associated with a duplication of target DNA at the site of insertion, leading to short, directly repeated sequences at the ends of the element. In pIP1206, only a single perfect duplication was found that flanked IS*1-5*. Thus, as previously proposed for the mosaic IncF p1658/97 plasmid (56), some of the mobile genetic elements have probably been acquired by recombination.

Antibiotic resistance genes. A 10-kb segment (G+C content, 45%), identical to a portion of pRSB107, contained genes responsible for tetracycline [*tet*(A)] and chloramphenicol (*catA*) resistance (Fig. 4A). As in pRSB107, the tetracycline resistance determinant (ORFs 104 to 107) was borne by a truncated derivative of Tn*10* bordered by two IS*1* sequences (IS*1-5* and IS*1-6*). The *tet* cluster is composed of four genes encoding a repressor (TetR), a tetracycline efflux antiporter [TetA(B)] belonging to the major facilitator superfamily, a transcriptional regulator (TetC), and a putative transcriptional regulator (TetD). Upstream from this cluster was *jemC* (encoding a putative repressor of arsenic and mercury resistance operons) and an ORF for a hypothetical protein (named YdjB in plasmid R100). In pIP1206, the *jemA*/*jemB* genes were not present, as opposed to Tn*10* (12). The *catA* gene (ORF 112) was located immediately after this truncated transposon. *ybjA* (ORF 113), which codes for an *N*-acetyltransferase, was downstream from *catA*.

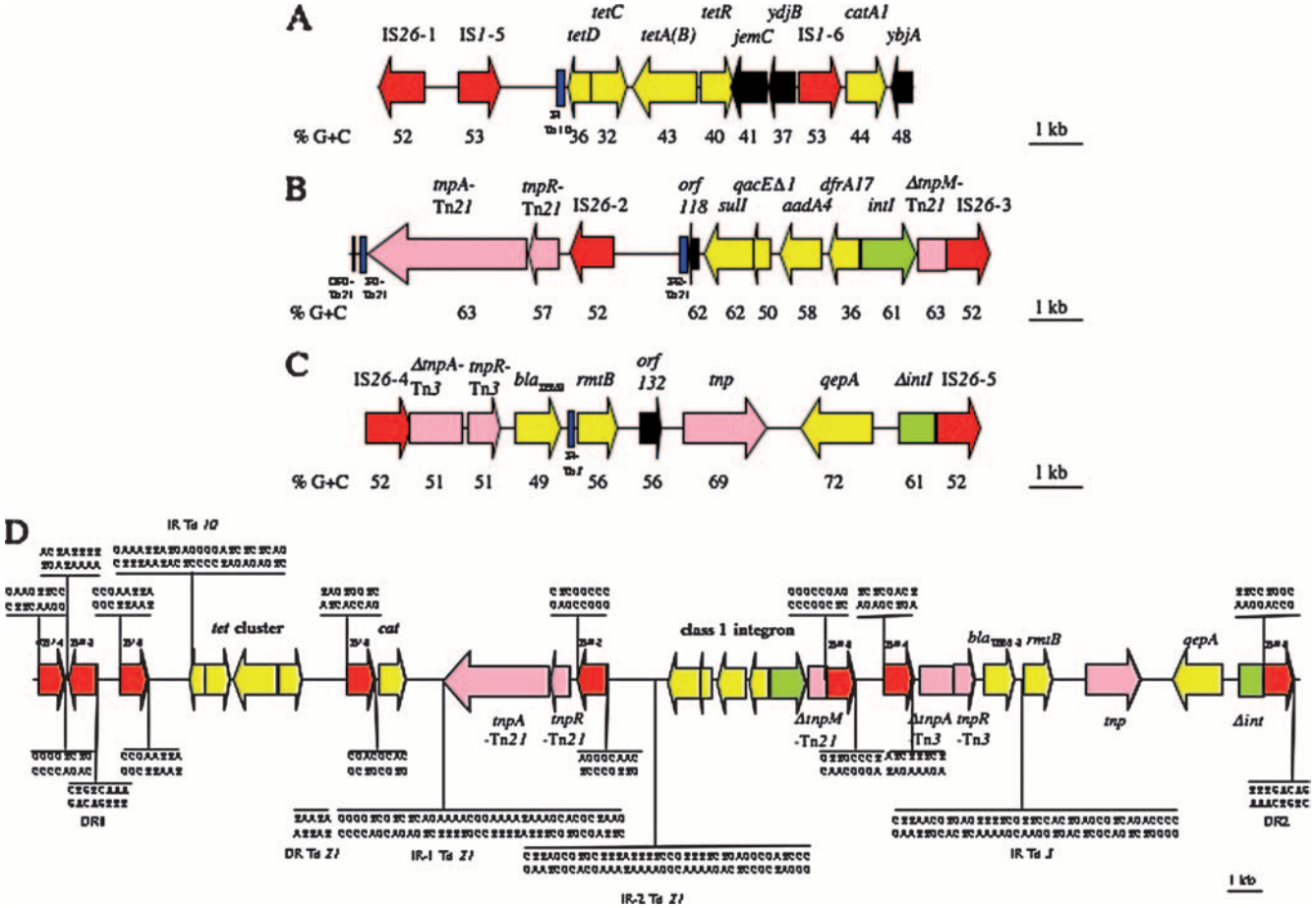

FIG. 4. Schematic representation of antibiotic resistance regions of pIP1206 to tetracycline and chloramphenicol (A), to sulfonamides, quaternary ammonium, streptomycin/spectinomycin, and trimethoprim (B), and to β-lactams, aminoglycosides, and fluoroquinolones (C). (D) Nucleotide sequences flanking the mobile elements. DR1 and DR2 flank at one end IS*26-1* and IS*26-5*. Genes for resistance are indicated in yellow, those for transposases/resolvases are in pink, integrases are in green, IS are in red, IRs and DRs are in blue, and ORFs for hypothetical proteins are in black. Arrows represent coding sequences and indicate the direction of transcription. The percent G+C content is indicated under the arrows. Truncated genes are indicated by a Δ symbol.

Downstream from the previous fragment, a remodelled Tn*21*-like transposon (ORFs 114 to 124; G+C content, 58%) was identified (Fig. 4B) (for a review, see reference 29). A Tn*21* derivative is also present in the pRSB107 antibiotic resistance region (47). The Tn*21*-like structure in pIP1206 was characterized by the presence of transposition genes (a truncated *tnpM*, *tnpR*, and *tnpA*) of a class I integron and by the absence of a set of genes encoding mercury resistance. The global structure of the transposon also differed from that in pRSB107. In pIP1206, direct repeat 1 (DR1), inverted repeat 1 (IR1), and the *tnpA-tnpR* genes were at the left end of the element. A 7.6-kb fragment, delineated by IS*26-2* and IS*26-3* and containing a second 38-bp IR (IR2), a class I integron, and the 3′-truncated regulator *tnpM*, constituted the remaining of the Tn*21*-like transposon. Curiously, this fragment was inverted relative to typical Tn*21*, *tnpM* being in the opposite orientation and at the right end of the transposon and IR2 being located between *tnpA* and *tnpM* (Fig. 4B). Analysis of the sequences flanking the IS*26* copies revealed that the 8-bp sequence downstream from IS*26-2* was identical to the 8 bp upstream from IS*26-3* and that the 8-bp sequence upstream from IS*26-2* was identical to the 8 bp downstream from IS*26-3* (Fig. 4D). Thus, recombination could have occurred between the two copies of IS*26*, leading to the final structure of the Tn*21*-like transposon in pIP1206. In summary, the evolution of this transposon could have occurred as follows: acquisition of IS*26-2* downstream from the Tn*21*-like transposon and of IS*26-3* within *tnpM*, followed by recombination between the two IS elements.

The class I integron (ORFs 119 to 123) is composed of *intI1* and four resistance genes: *sulI* (resistance to sulfonamides), *qacEΔ1* (to ammonium antiseptics), *aadA4* (to streptomycin and spectinomycin), and *dfrA17* (to trimethoprim) (Fig. 4B). The integron was identical to one located in the pEC1072 plasmid of *E. coli* EC107 and to another carried by a plasmid in *E. coli* 9516014-1 (13, 46) and similar to that of plasmid pAPEC-O2-R (95% identity at the nucleotide level), except for the presence of a *catB3* gene in pAPEC-O2-R.

Three additional resistance genes for a class A β-lactamase ($bla_{\text{TEM-1}}$), a methylase (*rmtB*), and an efflux pump (*qepA*) were located on an 11.3-kb fragment (ORFs 130, 131, and 134; G+C content, 59%) delineated by IS*26-4* and

IS*26-5* (Fig. 4C). RmtB is a 16S rRNA m^7G methyltransferase that modifies the N-7 position of nucleotide G1405 located in the A site of 16S rRNA and confers high-level resistance to all available aminoglycosides used for therapy, except streptomycin (43).

The *qepA* gene confers resistance to hydrophilic fluoroquinolones. The deduced sequence of QepA is 45% to 56% identical with various 14-transmembrane segment proton-dependent efflux pumps belonging to the MFS superfamily. The G+C content of *qepA* (72%) significantly differs from that of the chromosome of *Enterobacteriaceae* (50%), suggesting that *qepA* could have originated from actinomycetes (43, 54).

ORF133, having 99% identity with a putative transposase (accession number CT025832), was detected between the *rmtB* and *qepA* genes. Upstream from *qepA*, a gene for a class I integron integrase was disrupted by insertion of IS*26*-5 (Fig. 4C). A Tn*3*-like transposon, containing the bla_{TEM-1} gene, was present upstream from *rmtB* (Fig. 4C). The structural gene for the Tn*3* transposase was truncated due to insertion of IS*26-4*. A 38-bp sequence corresponding to an inverted terminal repeat typical of Tn*3* elements (accession number V00613) was found between bla_{TEM-1} and *rmtB* (Fig. 4D). A similar fragment containing bla_{TEM-1}, *rmtB*, and *qepA* has been reported in plasmid pHPA from an *E. coli* strain in Japan (54), suggesting that the association of these three resistance genes has a worldwide distribution.

In summary, all the antibiotic resistance genes were located within a 33.5-kb fragment delineated by IS*26-1* and IS*26-5* (Fig. 4D). IS*26* generates 8-bp DRs of target DNA upon transposition. Examination of the flanking sequences of each IS*26* copy revealed two identical DR sequences, one upstream from IS*26-1* (DR1) and the other downstream from IS*26-5* (DR2). However, the two IS*26* sequences were in opposite orientations (Fig. 4D), so that DR1 was located inside the 33.5-kb fragment. Of note, two IS*1* sequences (the 3′-truncated IS*1-4* and IS*1-5*) flanked IS*26-1*. One can thus propose that inversion of the IS*1-5*–DR1–IS*26-1*–ΔIS*1-4* fragment led to the sequence ΔIS*1-4*–IS*26-1*–DR1–IS*1-5*. Thus, this 33.5-kb fragment could (i) have been acquired from another replicon (plasmid or chromosome) by transposition or (ii) have resulted from successive integrations of mobile elements in a smaller fragment flanked by IS*26-1* and IS*26-5*.

Putative iron acquisition gene cluster. A fragment of 12 kb (ORFs 137 to 146; G+C content, 55%) flanked by IS*26-5* and IS*26-6* and identical to a segment of pRSB107 contained a putative iron acquisition system (47). This region is composed of genes for an iron permease, two integral membrane proteins, an ABC transporter, and a protein of the thioredoxin family. It has been suggested that pathogenic bacteria harboring plasmids with iron acquisition systems are more virulent (47).

***S*-Methylmethionine metabolism operon.** *S*-methylmethionine is an abundant plant product that can be used for methionine biosynthesis. An operon composed of *mmuP* and *mmuM* (ORFs 158 and 159; G+C content, 57%), two members of the methionine regulon, was present in pIP1206. MmuP is a permease involved in the uptake of *S*-methylmethionine, and MmuM is an *S*-methylmethionine:homocysteine methyltransferase that transfers a methyl group from *S*-methylmethionine to homocysteine to form two molecules of methionine (48). It has been proposed that expression of methyltransferase genes increases selenium tolerance and reduces nonspecific selenium incorporation into proteins (39).

Type I DNA restriction and modification system. R-M systems (i) protect the host genome from restriction by adding a methyl group on residues within specific target DNA sequences and (ii) restrict unmodified foreign DNA that penetrates into the cell (for a review, see reference 55). R-M enzymes have been classified into three types. The type I system, which recognizes two specific DNA sequences separated by a nonspecific spacer, contains genes coding for three subunits: HsdM, implicated in DNA methylation, HsdS, which determines DNA specificity, and HsdR, which is responsible for restriction. HsdM, HsdS, and HsdR (ORFs 163 to 165; G+C content, 44%) (Fig. 4) in pIP1206 exhibited, respectively, 99%, 43%, and 97% identity with the EcoR124/3-type IC R-M system (44) (Fig. 5A). DNA sequence specificity of the R-M endonuclease is ensured by HsdS, which is responsible for binding to the enzyme's DNA recognition site (18, 19, 44). This protein shared only a low degree of identity with known HsdS proteins, the highest level (57%) being with the R-M system of *Shewanella frigidimarina* (Table 1; Fig. 5A). It has been hypothesized that the type I HsdS consists of two specific domains, corresponding to the central and distal conserved regions, which recognize the two components of the target sequence (3). The sequence of HsdS of pIP1206 could be divided in five portions (Fig. 5B): part 1 (amino acids 1 to 18), part 3 (amino acids 156 to 227), and part 5 (amino acids 378 to 427) had a high degree of identity with HsdS of EcoR124/3 (from 94% to 100%), whereas part 2 (amino acids 19 to 155) and part 4 (amino acids 228 to 377) exhibited a higher level of identity with the R-M systems of *S. frigidimarina* and *Verminephrobacter eiseniae* (accession number CP000542), respectively. Amino acids 156 to 227 and 378 to 427 of HsdS of pIP1206 could represent the two specific domains. From these observations, we suggest that the specificity of the R-M system of pIP1206 could be a hybrid between those of *S. frigidimarina* and *V. eiseniae* and that the HsdS protein of pIP1206 has a common ancestry with that of EcoR124/3.

It has been argued that R-M systems can (i) be considered as selfish systems (25), (ii) mediate plasmid maintenance (27), and (iii) be part of mobile genetic elements (26). Thus, the $R\text{-}M_{pIP1206}$ system could also be implicated in stabilization of pIP1206 in the host strain. It is flanked by two IS fragments, which makes its mobility likely.

It has been shown that the sequence upstream from the *hsd* coding region of EcoR124/3 and EcoprrI, another type I R-M system, is conserved and composed of *doc* and *phd*, which are also present in the genome of bacteriophage P1 (50). The *phd* and *doc* genes encode an addiction system that stabilizes the P1 prophage:Doc, which is toxic to the host, with Phd as the antidote (28). The two genes (ORFs 161 and 162) also were present in pIP1206 upstream from *hsdM*. The deduced sequences shared 99% and 100% identity with Doc and Phd of P1, respectively. These genes are not present in *S. frigidimarina* and *V. eiseniae*.

Virulence-associated genes. A fragment of 8.6 kb (ORFs 171 and 172; G+C content, 47.4%) contained *vagC* and *vagD* (*v*irulence-*a*ssociated *g*enes). This portion was identical to a region of pAPEC-O1-ColBM, except for the presence in pIP1206 of an additional gene (ORF173) encoding a hypothetical protein.

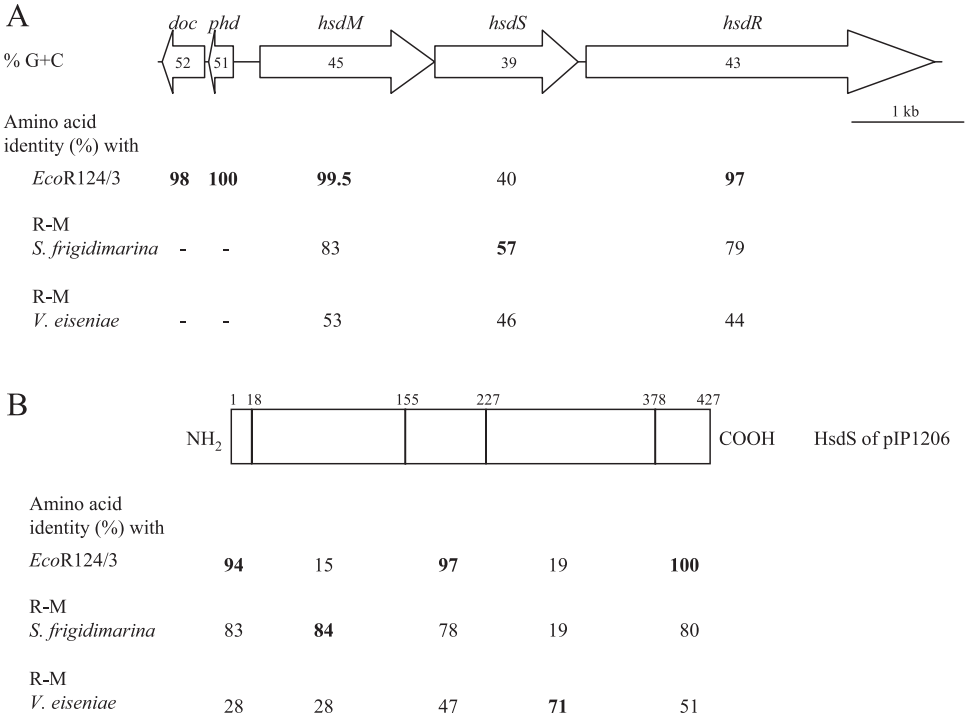

FIG. 5. Schematic representation of the 7-kb fragment containing the Hsd restriction-modification system (A) and HsdS of pIP1206 (B). The percent G+C content is indicated within the arrows. The highest amino acid identity is indicated in bold. A dash indicates absence of the gene.

The *vagC*/*vagD* locus is required for plasmid maintenance by coupling plasmid replication with cell division; VagD exerts the biological effect, whereas VagC regulates *vagD* expression (45). Upstream from this fragment, a segment of 2.3 kb (G+C content, 48%) contained ORFs 168 and 169, which encode two hypothetical proteins having 21% and 41% identity with proteins in *Vibrio splendidus*.

Concluding remarks. The entire sequence of pIP1206, isolated from an *E. coli* clinical strain, was determined because this plasmid carries two new genes for resistance to antibiotics: *qepA*, which confers resistance to hydrophilic fluoroquinolones by efflux, and *rmtB*, which directs production of a 16S rRNA methylase that confers high-level resistance to aminoglycosides (43). Analysis of the sequence revealed that pIP1206 contained a number of discrete functional elements. These subregions were delineated by insertion sequences, indicating that mobile elements play a major role in the mosaic structure of the replicon. (i) The main part of pIP1026 was probably derived from pRSB107, isolated from a bacterium in a wastewater treatment plant. (ii) Two IS*1* sequences flank the transfer region. DNA upstream and downstream from this region is common to pRSB107 and pAPEC-O1-ColBM. Thus, the *tra* region could have been acquired following homologous recombination between the two plasmids or by transposition. (iii) Of note, an RNA-directed DNA polymerase, present in two copies in pIP1206, had a high degree of similarity to a protein of *Shewanella* sp., a genus which is widespread in the environment and in wastewater. Furthermore, the *hsdS* gene of the restriction-modification system in pIP1206 was similar to that of *S. frigidimarina* and *V. eiseniae*. It therefore appears that pIP1206 was constructed by DNA exchange in the environment. (iv) All the antibiotic resistance genes were clustered in a fragment flanked by two IS*26* copies that contained transposons Tn*3*, Tn*10*, and Tn*21*. However, Tn*3* and Tn*10* had truncated transposase genes and Tn*21* had undergone several rearrangements, presumably due to sequential IS*26* insertions. Association of several antibiotic resistance genes with those for two efflux pumps in a plasmid that originated from a human clinical isolate but is also likely to be present in environmental bacteria represents a major risk for dissemination of multidrug resistance. (v) The stabilization of the plasmid in the host, and thus vertical inheritance of genetic information, is ensured by various types of maintenance systems: one active partition (*sop* operon), two postsegregational killing (*ccdA*/*ccdB* and *pemI*/*pemK* genes), and one restriction-modification (*hsd* genes) system.

In summary, pIP1206 may have resulted from recombination between pRSB107 and a pAPEC-01-ColBM-like plasmid associated with structural rearrangements following acquisition of additional DNA by recombination and of mobile elements by transposition; its stabilization is due to the presence of four different systems.

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