Complete Nucleotide Sequence of the Conjugative Tetracycline Resistance Plasmid pFBAOT6, a Member of a Group of IncU Plasmids with Global Ubiquity

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This study presents the first complete sequence of an IncU plasmid, pFBAOT6. This plasmid was originally isolated from a strain of *Aeromonas caviae* from hospital effluent (Westmorland General Hospital, Kendal, United Kingdom) in September 1997 (G. Rhodes, G. Huys, J. Swings, P. McGann, M. Hiney, P. Smith, and R. W. Pickup, Appl. Environ. Microbiol. 66:3883–3890, 2000) and belongs to a group of related plasmids with global ubiquity. pFBAOT6 is 84,748 bp long and has 94 predicted coding sequences, only 12 of which do not have a possible function that has been attributed. Putative replication, maintenance, and transfer functions have been identified and are located in a region in the first 31 kb of the plasmid. The replication region is poorly understood but exhibits some identity at the protein level with replication proteins from the gram-positive bacteria *Bacillus* and *Clostridium*. The mating pair formation system is a *virB* homologue, type IV secretory pathway that is similar in its structural organization to the mating pair formation systems of the related broad-host-range (BHR) environmental plasmids pIPO2, pXF51, and pSB102 from plant-associated bacteria. Partitioning and maintenance genes are homologues of genes in IncP plasmids. The DNA transfer genes and the putative *oriT* site also exhibit high levels of similarity with those of plasmids pIPO2, pXF51, and pSB102. The genetic load region encompasses 54 kb, comprises the resistance genes, and includes a class I integron, an IS630 relative, and other transposable elements in a 43-kb region that may be a novel Tn1721-flanked composite transposon. This region also contains 24 genes that exhibit the highest levels of identity to chromosomal genes of several plant-associated bacteria. The features of the backbone of pFBAOT6 that are shared with this newly defined group of environmental BHR plasmids suggest that pFBAOT6 may be a relative of this group, but a relative that was isolated from a clinical bacterial environment rather than a plant-associated bacterial environment.

The genus *Aeromonas* comprises species that occupy a wide variety of habitats and has members that are ubiquitous in most aquatic environments (19). Numerous species, including *Aeromonas salmonicida*, *Aeromonas hydrophila*, and *Aeromonas veronii* (4), have been implicated in fish diseases, and some species (*A. hydrophila*, *A. veronii*, *Aeromonas jandaei*, *Aeromonas trota*, and *Aeromonas schubertii*) have been implicated in pathogenicity in humans (25). Antimicrobial agents have been used extensively for prevention and treatment of human and fish diseases. This has resulted in an increase in bacterial resistance to antibiotics to the extent that treatment of human and fish diseases may have been compromised (54). Next to the spread of successful clonal lineages of resistant strains, horizontal transfer of resistance genes between bacteria by broad-host-range (BHR) plasmids is largely responsible for the dissemination of this resistance. In tetracycline-resistant *Aeromonas* species, particularly *A. salmonicida*, incompatibility group U (IncU) plasmids have been shown to be very common,

and recently it has been demonstrated that these R plasmids form a very closely related group of plasmids that have a common backbone (1, 45, 57). IncU tetracycline resistance plasmids belonging to the pASOT group (1) and the pFBAOT group (45) and plasmids pRAS1 and pAr-32 (49) have been the main focus of these studies. Tn1721-like transposons which carry the TetA tetracycline resistance determinant and class I integrons that carry and can mobilize additional resistance genes on cassettes have been found in many but not all plasmids in this family (29, 51, 57). The resistance genes that have been shown to be carried by integrons in this family of IncU plasmids are *aadA1* and *aadA2* (streptomycin resistance), *sulI* and *sulII* (resistance to sulfonamides), *dfrA16* and *dfrIIc* (*dfrB3*) (trimethoprim resistance), and *catAII* (chloramphenicol resistance) (57). The ranges of environments, geographical locations, and bacterial species in which tetracycline-resistant IncU plasmids have been found have led to the hypothesis that the aquaculture and human compartments of the environment are interactive and behave as a single compartment (45). However, this plasmid group is still relatively poorly understood, and to date no IncU plasmids have been sequenced in their entirety. Consequently, core functions, such as the replication, maintenance, and transfer functions, remain undescribed. In

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this paper we describe the first DNA sequence of an IncU plasmid, plasmid pFBAOT6. This plasmid was originally isolated from a hospital effluent (Kendal, Cumbria, United Kingdom) strain of *Aeromonas caviae* HG5B in September 1997 and was subsequently transferred to *Escherichia coli* J53-1 by conjugation (21, 45).

MATERIALS AND METHODS

Bacterial strain. *E. coli* J53-1(pFBAOT6) was cultured as described previously (45).

DNA extraction and sequencing. pFBAOT6 plasmid DNA was extracted with a QIAGEN plasmid midi kit used according to the manufacturer's instructions (QIAGEN, West Sussex, United Kingdom). DNA from pFBAOT6 was sonicated for 10 s in a VirSonic 300 sonicator (Virtis Co. Inc., Gardiner N.Y.) fitted with a cup horn probe. The sonicated material was treated with mung bean nuclease (Amersham) and was size fractionated by agarose gel electrophoresis. Fragments in the size range from 1.4 to 2 kb were ligated into pUC18 which had been digested with SmaI and treated with bacterial alkaline phosphatase (Q-Biogene). Ligated DNA was transformed into *E. coli* DH10B Electromax cells (Invitrogen) by using a Bio-Rad GenePulser. In total, 2,134 end sequences, giving approximately 11-fold coverage of the plasmid, were generated from this library by using ABI Big-Dye terminator chemistry with ABI3730 automated sequencers. These sequences were assembled by using phrap (<http://www.phrap.org/>) and were finished by using the GAP4 software (8). The final assembly was verified by comparisons with EcoRI, HindIII, and BamHI digests of the original plasmid. The consensus sequence had a quality score of >30 at each base (equivalent to an estimated error rate of <1 bp per 1.73 Mb).

Sequence analyses. The sequence was annotated by using Artemis (46). Predicted coding sequences (CDSs) were identified manually with reference to positional base composition and amino acid usage plots. The entire sequence was searched in all six reading frames against the nonredundant TrEMBL database by using BLASTX (3) to ensure that no genes were missed. Each CDS was searched against the nonredundant databases by using FASTA (43) and BLASTP (3) and against the PFAM (6) and Prosite (20) databases of protein motifs. Transmembrane helices were identified with TMHMM (27), and signal sequences were identified with SignalP (39). Repeats were identified by using Dotter (56). Multiple-sequence alignments were constructed by using ClustalW (60) and were shaded by using BOXSHADE (www.ch.embnet.org).

Nucleotide sequence accession number. The sequence and annotation have been deposited in the EMBL/GenBank database under accession number CR376602.

RESULTS AND DISCUSSION

General features of pFBAOT6. pFBAOT6 is 84,748 bp long and has an overall G+C content of 57%. It contains 94 CDSs, 82 of which encode proteins with homology to sequences in databases (Table 1 and Fig. 1). DNA sequence analysis has confirmed the majority of preliminary structural observations for pFBAOT6 made by EcoRI restriction fragment length polymorphism analyses and hybridization to specific DNA probes (45). Genes predicted to encode replication, maintenance, and transfer proteins are located in the first 31 kb of the plasmid, while the remaining 54 kb contains accessory regions that include most of the genes with no attributed functions.

Putative replication functions. The numbering of pFBAOT6 commences at the first nucleotide of the ATG start codon of pFBAOT6.01, a gene predicted to encode a replication protein. The putative replication protein (Rep) comprises 459 amino acids (aa) and contains a predicted helix-turn-helix (H-T-H) motif near the C terminus (Fig. 2). The highest level of similarity at the amino acid level is the level of similarity with the putative replication protein (Rep43) from a cryptic plasmid found in *Bacillus* sp. strain KSM-KP43 (47). The Rep protein is also related to the replication protein encoded by plasmid

pIP404 in *Clostridium perfringens* CPN50 (16) and to the nearly identical putative replication proteins (CTP1 and CTP45) encoded by *Clostridium tetani* plasmid pE88 (9). The sizes of CTP1 and CTP45 are virtually identical to the size of Rep (460 and 461 aa, respectively), while the Rep proteins from the cryptic plasmid in *Bacillus* sp. strain KSM-KP43 and plasmid pIP404 are 432 and 406 aa long, respectively. Alignment of pFBAOT6 Rep with these related Rep proteins revealed several conserved regions that occupy mainly the central region of the protein (Fig. 2). The amino- and carboxy-terminal regions appear not to be highly conserved. Like the replicative origins of plasmid pE88 and the cryptic plasmid from *Bacillus* sp. strain KSM-KP43, the replicative origin of pFBAOT6 remains unclear. The origin of pIP404 has been proposed to consist of several AT-rich 8- to 10-bp iteron repeats (16). There are no such regions close to *rep* in pFBAOT6, although upstream of the start codon (between positions 83855 and 84951) there is a series of three identical 98-bp direct repeats followed by a truncated version of the same repeat. These repeats have some internal similarity to a series of five complete (80-bp) and two incomplete (33-bp) direct repeats situated downstream of the gene (between positions 1523 and 2073). Although the role of these related flanking repeats is unknown, their proximity to the putative replication protein may imply a role in regulation or replication. Previous analyses of EcoRI-digested DNA of pFBAOT6 and related IncU plasmids showed that the EcoRI-derived IncU probe (approximately 950 bp) hybridized to a region that was a similar length (45). Sequencing has shown that only two EcoRI fragments could have hybridized to the IncU probe. One of these fragments occupies the region between bases 84325 and 537 (960 bp) and overlaps the proposed *rep* gene. This is consistent with the hypothesis that it also is a region associated with plasmid incompatibility, as the original 950-bp EcoRI fragment conferred incompatibility upon the vector into which it was cloned (pULB2130) (12). The other EcoRI fragment that was a similar length (958 bp) is located between positions 77338 and 78296, spanning a region that encodes putative proteins with homology to proteins encoded by *Delftia acidovorans* plasmid pUO1 (58) and the truncated transposase of Tn1721. Clearly, the *rep* region in pFBAOT6 is of interest for future study as it is probably present to some degree in all IncU plasmids.

Maintenance and partitioning. The stable inheritance of plasmids is dependent on several control features that prevent irreversible plasmid loss during cell division and growth. In IncP plasmids these control mechanisms are known to include centromere-like partitioning systems, postsegregational killing systems, and plasmid-encoded restriction-modification systems (17). In pFBAOT6, genes proposed to encode maintenance and partition proteins are clustered together in a region downstream of the *rep* gene (pFBAOT6.03 and pFBAOT6.05 to pFBAOT6.10). These genes have counterparts in IncP plasmids that correspond to *klaA*, *korC*, *traN*, *kfrA*, *korA* (*trfB*), *incC2*, and *korB*.

pFBAOT6.03 encodes a 141-aa protein that is most closely related (41% identity) to the IncP-1 β plasmid antirestriction protein KlcA (although in IncP-1 β plasmids no antirestriction activity has been detected for this protein [30]). In IncP plasmids *klaA* is part of the *kilC* operon (*klaA-klaB-korC*) and has no known function (7). In *E. coli* expression of the *kilC* operon

TABLE 1. Predicted genes in plasmid pFBAOT6

CDS	Name	Position	Protein length (aa)	Direction	G + C content (%)	Amino acid identity to informative database match (accession no.)
1	<i>rep</i>	1–1380	459	→	55.36	30.79% in 354 aa; replication protein Rep43 (AB034994)
2	pFBAOT6.02	2248–2526	92	→	53.05	Hypothetical protein with no significant database matches
3	<i>klcA</i>	2637–3062	141	→	53.75	41.25% in 143 aa; IncP-β plasmid KlcA/KilC (P52602)
4	pFBAOT6.04	3190–3342	51	→	56.21	Hypothetical protein with no significant database matches
5	<i>korC</i>	3391–3687	98	→	51.85	50% in 92 aa; plasmid RK2 KorC (Q52331)
6	<i>traN</i>	3692–4759	355	→	55.14	57.1% in 331 aa; plasmid RP4 TraN (Q60216)
7	<i>kfrA</i>	4889–5956	355	→	57.68	32.86% in 356 aa; plasmid R751 KfrA (U67194)
8	<i>korA</i>	6010–6348	112	→	56.34	36% in 100 aa; IncP-β plasmid KorA or TrIB (Q57423)
9	<i>incC2</i>	6353–7105	250	→	52.86	40.85% in 257 aa; plasmid pB10 IncC2 (AJ564903)
10	<i>korB</i>	7102–8460	452	→	59.82	32.52% in 452 aa; plasmid R751 KorB (Q52313)
11	pFBAOT6.11	8441–8749	102	→	55.66	Hypothetical protein with no significant database matches
12	<i>mpR</i>	8816–9547	243	←	53.82	50% in 254 aa; plasmid pKM101 Zn metalloprotease MpR (U72482)
13	<i>mobC</i>	9837–10367	176	→	54.42	30.35% in 168 aa; plasmid pIPO2 hypothetical protein (AJ297913)
14	<i>virD2</i>	10360–11355	331	→	54.91	40.75% in 346 aa; plasmid pIPO2 TraR (AJ297913)
15	<i>traC3</i>	11394–13088	564	←	52.80	41.31% in 593 aa; IncP plasmid DNA primase TraC3 (U67194)
15A	<i>traD</i>	13094–13237	48	←	50.00	48.78% in 41 aa; IncP plasmid DNA primase TraD (P27193)
16	<i>traC4</i>	13227–16292	1021	←	52.90	35.57% in 357 aa; IncP plasmid DNA primase TraC4 (P27189)
17	<i>virD4</i>	16305–18230	641	←	47.40	31.5% in 600 aa; <i>Agrobacterium tumefaciens</i> VirD4 (P09817)
18	<i>virB11</i>	18196–19188	330	←	48.53	49.21% in 319 aa; plasmid p42d VirB11 (U80928)
19	<i>virB10</i>	19202–20416	404	←	56.87	43.42% in 403 aa; plasmid pIPO2 TraL (AJ297913)
20	<i>virB9</i>	20416–21249	277	←	48.56	39.65% in 290 aa; plasmid pIPO2 TraK (AJ297913)
21	<i>virB8</i>	21252–22001	249	←	54.13	39.09% in 243 aa; plasmid pIPO2 TraJ (AJ297913)
22	pFBAOT6.22	22004–22207	68	←	55.39	35% in 60 aa; <i>Brucella suis</i> 12 protein (AF141604)
23	<i>virB6</i>	22322–23332	336	←	54.59	28.57% in 315 aa; plasmid pSymA VirB6 (AE007259)
24	pFBAOT6.24	23344–23613	89	←	54.81	39.02% in 82 aa; plasmid pIPO2 hypothetical protein (AJ297913)
25	<i>virB5</i>	23648–24286	212	←	55.08	28.63% in 220 aa; <i>Brucella abortus</i> VirB5 (AF226278)
26	<i>virB4</i>	24289–26736	815	←	50.65	42.14% in 821 aa; plasmid pIPO2 VirB4 (AJ297913)
27	<i>virB3</i>	26733–27053	106	←	48.90	32.65% in 98 aa; plasmid p42d VirB3 (U80928)
28	<i>virB2</i>	27071–27394	107	←	55.55	30.2% in 96 aa; plasmid pSymA VirB2 (AE007259)
29	pFBAOT6.29	27372–27728	118	←	41.73	50.63% in 79 aa; plasmid pXF51 protein Xfa0004 (AE003851)
30	<i>top</i>	27793–29769	658	←	55.99	56.75% in 666 aa; plasmid pXF51 DNA topoisomerase 1 (AE003851)
31	pFBAOT6.31	29766–30503	245	←	47.15	27.32% in 172 aa; phage PBC5 hypothetical protein 33 (AF448724)
32	pFBAOT6.32	30530–31561	343	←	52.51	62.09% in 343 aa; IS630 family transposase (P16943)
33	pFBAOT6.33	31674–32189	171	←	54.65	28.08% in 146 aa; plasmid pSD25 protein Rc113 (AF416331)
34	pFBAOT6.34	32353–32742	129	→	48.71	Putative integral membrane protein with no significant database matches
35	pFBAOT6.35	32762–33019	86	→	44.57	Hypothetical protein with no significant database matches

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TABLE 1—Continued

CDS	Name	Position	Protein length (aa)	Direction	G + C content (%)	Amino acid identity to informative database match (accession no.)
36	pFBAOT6.36	33015–33410	131	→	42.17	29.26% in 123 aa; LysR family transcriptional regulator (BX640416)
37	<i>resA</i>	33478–34044	189	←	56.43	100% in 127 aa; pRAS1 truncated resolvase (AJ517790)
39	<i>tnpA</i>	34336–35130	264	←	61.00	100% in 264 aa; pRAS1 IS6100 transposase (AJ517790)
40	<i>orf6</i>	35229–35583	95	←	61.05	100% in 95 aa; pRAS1 ORF6 hypothetical protein (AJ517790)
41	<i>orf5</i>	35607–36107	166	←	65.06	100% in 95 aa; pRAS1 ORF5 hypothetical protein (AJ517790)
42	<i>sull</i>	36235–37074	279	←	61.66	100% in 279 aa; Sull (AJ517790)
43	<i>qacEΔ1</i>	37068–37415	115	←	50.00	100% in 115 aa; QacEdelta 1 (AJ517790)
44	<i>aadA2</i>	37579–38370	263	←	52.02	100% in 263 aa; streptomycin adenylyltransferase protein (AF227505)
45	<i>int1</i>	38516–39529	337	→	61.14	100% in 337 aa; plasmid pRAS1 integrase/recombinase (AJ517790)
46	<i>res</i>	39878–40444	188	←	67.37	95.72% in 187 aa; plasmid pXAC64 resolvase (AE008925)
47	pFBAOT6.47	40573–40821	82	←	61.44	71.01% in 69 aa; plasmid pB10 hypothetical protein Xf2080 (AJ564903)
48	<i>orf1</i>	41192–42769	525	←	47.52	100% in 525 aa; Tn1721 methyl-accepting chemotaxis protein (X61367)
49	<i>tnpR</i>	43079–43639	186	→	65.59	100% in 186 aa; Tn1721 and Tn4653 TnpR resolvase (P06692)
50	<i>tnpAΔ1</i>	43643–46531	963	→	65.92	98.66% in 973 aa; truncated Tn1721 transposase TnpA' (P51565)
51	<i>tnpA</i>	46551–47825	424	←	50.27	38.97% in 408 aa; plasmid p37 ISSBI transposase (AJ305328)
52	pFBAOT6.52	47982–48353	123	→	59.13	Hypothetical protein with no significant database matches
53	<i>tnpA</i>	48671–49939	422	→	56.34	37.04% in 409 aa; plasmid p37 ISSBI transposase (AJ305328)
54	pFBAOT6.54	50549–51559	336	→	61.32	64.98% in 337 aa; putative oxidoreductase transmembrane protein (AL591785)
55	pFBAOT6.55	51633–52064	143	→	62.50	44.36% in 142 aa; <i>Pseudomonas putida</i> conserved hypothetical protein (AE109307)
56	pFBAOT6.56	52127–52414	96	→	61.11	38.37% in 86 aa; putative oxidoreductase transmembrane protein (AL591783)
57	pFBAOT6.57	52569–53039	156	→	61.78	35.13% in 148 aa; <i>Bordetella parapertussis</i> hypothetical protein (BX640427)
58	pFBAOT6.58	53043–53555	170	→	63.15	46% in 150 aa; <i>Yersinia pestis</i> hypothetical protein (AJ414160)
59	pFBAOT6.59	53552–54814	420	→	64.44	37.59% in 391 aa; transmembrane transport protein (AE012416)
60	pFBAOT6.60	54845–55246	133	←	57.21	25.39% in 126 aa; <i>Pseudomonas syringae</i> endoribonuclease L-PSP family (AE016864)
61	pFBAOT6.61	55353–55802	149	←	57.77	28.34% in 127 aa; transcription elongation factor (AE014724)
62	pFBAOT6.62	55890–56462	190	←	58.81	Hypothetical protein with no significant database matches
63	<i>corA</i>	56554–57522	322	←	59.33	54.96% in 322 aa; Mg and Co transport protein CorA (AE005982)
64	pFBAOT6.64	57519–58223	234	←	60.28	64.05% in 242 aa; glutathione S-transferase family protein (AE005806)
65	<i>msrA</i>	58356–58895	179	←	62.22	76.68% in 163 aa; peptide methionine sulfoxide reductase (P96814)
66	<i>msrB</i>	58897–59352	151	←	59.42	78.08% in 146 aa; peptide methionine sulfoxide reductase (Q92Y46)
67	pFBAOT6.67	59487–60362	291	←	59.47	61.16% in 291 aa; LysR family transcriptional activator (AE007290)
68	pFBAOT6.68	60591–61823	410	→	65.20	44.49% in 409 aa; hypothetical protein (AE007290)
69	<i>msrA2</i>	61856–62419	187	→	58.86	64.11% in 170 aa; peptide methionine sulfoxide reductase (P96814)

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TABLE 1—Continued

CDS	Name	Position	Protein length (aa)	Direction	G + C content (%)	Amino acid identity to informative database match (accession no.)
70	pFBAOT6.70	62419–63444	341	→	64.23	30.08% in 339 aa; enoyl reductase LovC (AF141925)
71	pFBAOT6.71	63841–64380	179	←	59.07	37.5% in 160 aa; MarR family transcriptional regulatory protein (AP006577)
72	pFBAOT6.72	64652–65059	135	←	58.08	Hypothetical protein with no significant database matches
73	pFBAOT6.73	65102–65599	165	←	54.81	43.62% in 149 aa; <i>Bradyrhizobium japonicum</i> protein (AP005958)
74	pFBAOT6.74	65863–66249	128	→	58.65	Hypothetical protein with no significant database matches
75	pFBAOT6.75	66288–67247	319	←	58.02	Hypothetical protein with no significant database matches
76	pFBAOT6.76	67274–67807	177	←	59.55	35.37% in 147 aa; MarR family transcriptional regulator (AP006577)
77	pFBAOT6.77	68064–68960	298	→	59.64	30.95% in 210 aa; putative LysR-type transcriptional regulator (AF261825)
78	<i>trbI</i>	69196–70416	406	←	67.32	43.91% in 419 aa; Tn4371 conjugal transfer protein TrbI (Y10832)
79	<i>trbG</i>	70413–71462	349	←	65.80	58.28% in 326 aa; conjugal transfer protein TrbG (AP003009)
80	<i>trbF</i>	71459–71779	106	←	66.66	80.43% in 92 aa; pMLb conjugal transfer protein TrbF (AP003017)
81	<i>tnpA</i>	71813–74779	988	←	65.58	92.3% in 988 aa; Tn21 transposase TnpA (P06694)
82	pFBAOT6.82	74932–75228	98	→	63.63	98.95% in 96 aa; plasmid pUO1 ORF6 protein (AB063332)
83	pFBAOT6.83	75225–75611	128	→	60.46	99.2% in 125 aa; plasmid pUO1 ORF6 protein (AB063332)
84	pFBAOT6.84	75627–75797	57	→	63.74	Hypothetical protein with no significant database matches
85	pFBAOT6.85	75848–76201	118	→	53.10	100% in 118 aa; plasmid pUO1 ORF7 protein (AB063332)
86	pFBAOT6.86	76459–76785	108	→	66.66	100% in 108 aa; plasmid pUO1 ORF8 protein (AB063332)
87	pFBAOT6.87	76782–77282	166	→	64.47	100% in 153 aa; plasmid pUO1 ORF9 protein (AB063332)
88	pFBAOT6.88	77279–77650	123	→	63.17	100% in 123 aa; plasmid pUO1 ORF10 protein (AB063332)
89	<i>invA</i>	77644–78201	185	→	63.62	100% in 185 aa; plasmid pUO1 DNA invertase InvA (AB063332)
90	<i>tccA</i>	78315–78575	88	→	70.45	91.04% in 67 aa; Tn1721 relaxase/helicase TccA (P03038)
91	<i>tetR(A)</i>	78613–79263	216	←	63.90	100% in 216 aa; Tn1721 TetR(A) (P03038)
92	<i>tet(A)</i>	79369–80568	399	→	63.66	100% in 399 aa; Tn1721 TetA(A) (P02982)
93	<i>pecM</i>	80600–81484	294	←	58.98	100% in 294 aa; plasmid pB10 PecM-like protein (AJ564903)
94	<i>tnpA'</i>	82018–83769	583	→	65.58	99.82% in 583 aa; Tn1721 truncated transposase TnpA' (P51565)

is lethal unless it is regulated by KorA and KorC (30). Interestingly, the pFBAOT6 *kbcA* gene promoter region is part of a 55-bp imperfect repeat sequence that also occurs before the previous gene (pFBAOT6.02). This repeat includes –10 and –35 sites, ribosome and transcription factor binding sites, and inverted repeats (IRs) (which are often associated with transcription regulation), and it ends 10 bp downstream of the start codon (data not shown).

pFBAOT6.05 encodes a KorC homologue (50% identity with KorC_{RRK2}) which contains a probable transmembrane helix. pFBAOT6.03 and pFBAOT6.05 are separated by a gene

with an unknown function (pFBAOT6.04) that encodes a predicted 5.7-kDa protein that has no database matches.

pFBAOT6.06 is similar to *traN* in RP4 (57% identity), while the predicted pFBAOT6.07 gene product is a KfrA homologue which in IncP-1 plasmids and the wheat rhizosphere plasmid pIPO2 is assumed to be nonessential as it autoregulates an operon that provides auxiliary maintenance functions (59). Expression of KfrA_{pFBAOT6} may be regulated as the gene is preceded by inverted repeats and a putative operator in the –10 promoter region (data not shown). KfrA_{pFBAOT6} is larger than its counterparts due to the presence of a 105-bp sequence

pFBAOT6	1	-----MAQ-----AQLSLFGQN-----EATYHDTSAAGFFSLLVQWP-TGKRQSSFP
KP-43	1	-----MEN-----ANSIQKAG-----YHAWFOHSDAD-GWITVARKGESNSFIOKHYKP
pE88	1	MLAKQLSLYDYIDNTLKNLVTCVEEIKAEEDNIQKATFHMDTLHKGDG-GYITIAIKHN-NDWSQYHYKI
C. tetani	1	MLAKQLSLYDYIDNTLKNLVTCVEEIKAEEDNIQKATLHMDTLHKGDG-GYITIAIKHN-NDWACHHYKI
pIPO4	1	-----MRN-----ALKLNKKN-----YIDNIHSRSK-GWITRSVIDK-KGYSQWHYKY
pFBAOT6	44	EOMPT---VLNAVDRHLDTWLSQAEFMRPNRRVNVNLLRIGLFAADLDTVKMPWAQG--RKPEELAAAITY
KP-43	44	TELAD---KLTEWLG-EDVFFSONTFYRPORSIENVRLRSLYTDLDEYLFNYDP---SWVMGKLOHEF
pE88	69	EELKENVGKVLSDG-VNIYLSFNSFYKPIRRIENVRLNSLYIDLDYITLDKFKDLTVQDILWNLEADY
C. tetani	69	EELKDNVGKVLSDG-VNIYLSFNSFYKPIRRIENVRLNSLYIDLDYITLDKFKDLTVQDILWNLEADY
pIPO4	42	AELKD----LDMSD-ENIYITLNTFYKPCRRRIENIKELNLTLEIDLDYKTKGKTK---DQVLMLEKNY
pFBAOT6	109	FCQSEGIPQPSIMVYSGRGIQAKWLLERPVRQALPRWNACQTHLINKLAELGADPGAKDASRVLRVNT
KP-43	106	FGQS--IPEPNLIFSGGGIVLILWLLD-PVPHKALPLWQAVONHFLSQLEELGGDPKAADAARVFRAGS
pE88	138	FKSK--VPEANFIVITGRGLAIYWLIE-AVPYKALPLWNAVQKNFLNKLKEIGADEKSIDAARVMRLSGS
C. tetani	138	FKSK--VPEANFIVITGRGLAIYWLIE-AVPYKALPLWNAVQKNFLNKLKEIGADEKSIDAARVMRLSGS
pIPO4	102	FNQS--IPIPNYVIDSGRGMYLIIIN-AVPSKALPLWKAVQGEYLYNQLKYFGADRQALDATRILRVPGS
pFBAOT6	179	VNSKSGAVCRVVHLEEGGLPIRYNFEYLAEMLPVARWDIEKOKAERQOARLTVNGGKVGNNLRTSGR
KP-43	173	VNSKNGAEVRAEFRHD-----HKYQLRQLQDYLPDELNDI-NPPKKKKRGRKKRVQ-----LFNTY
pE88	205	VNQKNGHAVDLLFYND-----NKNYLRDIQENYLPDLTPYV-KNPYHKAKGRKKRVN-----LFNLY
C. tetani	205	VNQKNGHAVDLLFYND-----NKNYLRDIQENYLPDLTPYV-KNPYHKAKGRKKRVN-----LFNLY
pIPO4	169	INSKSKTVVNIIDEYE-----YIYDLREIQNGELPELK-----PYERKKGRPSKINY-----TYRER
pFBAOT6	249	QLAWDRLEDLRKLGQLRG-----GINEGERMRHLFWRLNELLGSGATNSAQMHEAAALSSELDTR
KP-43	230	KLHYARLMDLVRLVELRN-----YQVKGHRELICFLYR-YWQCCYSNDPSEALNQTMTLNLQESAP
pE88	262	SLHYARLRLDLVKLMELREGMCRMEDGSLKATGLRELFMCFLYR-YWSSCYERDTENALQSTLDFNKQFREP
C. tetani	262	SLHYARLRLDLVKLMELREGMCRMEDGSLKATGLRELFMCFLYR-YWSSCYEGDIENALKSTLDFNKQFREP
pIPO4	221	SLVYGRIDIIKICELRE-----YDLKGHRELILFLYR-YVLCSETEDIEKALNDVLELNSMERQH
pFBAOT6	310	WSYRSKELMTLYSKAKSYEAGE-----RVEFGGKSFAPLYTFKNDTLINLFOISQOEQEQLKTIISTDEA
KP-43	290	LITREVERATR-SAEKAWERNNEEANKIAIDKGYPGAGYINISNKKLISWLDISSDEMTHLSTIIDGLEK
pE88	331	LNISEVEKATN-SAQKAYKAWL-----KDCPTGTYSRGGYNYKNKTLIKLLNITEDEMKVLETIIDNKEV
C. tetani	331	LSIGEVERATK-SATKAYKQWL-----KDCPTGTYSRGGYNYKNKTLIKLLNITEDEMKVLETIIDNKEV
pIPO4	281	LSREVRITRATR-SAERCYLDKN-----K-----Q-----YKYKNETLIEELLEITEEBQYMTIIISKEY
pFBAOT6	375	QKRRREEDRLRDEER-RRAGQLEREAYEANSLSRQKPWEAMGMSRAKWYRLGKPSPOQNSSETSPSPITN
KP-43	359	NRKRRTANMEMRREGVVKPREYIIEOHAKTDNQLTNLQALQE-----NPKAKRRDLAATIGVSV
pE88	395	KRRNTNIRTNKHHKAK-RRNEKGLTKRQOEKEN--TIMLVKKLLK-----KG-LGVKAIARELNINI
C. tetani	395	KRRDNEKNKARQKAK-RRNELGLTKRQOEKEN--KIREVKELYN-----KG-LTOKEIANKLDITD
pIPO4	335	KRRENIRGKKNYQEQ-LKAKGKATKKE-ELNV--LRKKIKALKE-----KG-FKNKEITLMLEVPI
pFBAOT6	444	GEASAPALQAAACKAV
KP-43	420	FRIDQLKRQLKSL---
pE88	452	SAVSR-TKNNKY---
C. tetani	452	RYVRK-ILNKK----
pIPO4	391	KTLERHITYMKNGLL

FIG. 2. Multiple-sequence alignment of pFBAOT6 replication protein with its closest relatives from *Bacillus* sp. strain KSM-KP43 (KP-43), plasmid pE88, *C. tetani*, and plasmid pIPO4. A black background indicates amino acids that are fully conserved, while a gray background indicates amino acids that are 50% conserved. The bar above the sequences indicates the helix-turn-helix motif for Rep_{pFBAOT6}.

that is repeated 4.5 times toward the 3'-OH end of the gene. This results in repetition of a 35-amino-acid sequence in the carboxy-terminal half of the protein.

pFBAOT6.08, pFBAOT6.09, and pFBAOT6.10 of this region correspond to the *incC-korB* (*korA-incC2-korB*) gene cluster found in the central control region (CtI) of IncP-1 β plasmids (41). The IncC and KorB proteins interact and are homologues of partition proteins belonging to the ubiquitous ParA and ParB families, respectively (17). ParB proteins are DNA binding proteins and usually contain a putative H-T-H motif. In plasmid RK2, KorB interacts with IncC in plasmid partitioning and is a global regulator of transcription that can bind at centromere-like sequences (operators) that occur at 12 distinct sites in the plasmid. By binding operators KorB_{RK2} can coordinate expression of at least five operons that code for stable inheritance and transfer. The KorB operator sequence (*O_B*) is highly conserved at TTTAGCSGCTAAA (41) and has

recently been split into two classes based on the location in relation to promoter sequences. Class I *O_B* sequences are situated 4 bp upstream of the -35 promoter region, while class II *O_B* sequences are located between 80 and 189 bp from the transcription start point (24). Interestingly, pFBAOT6 does not contain any sequences that correspond to the *O_B* consensus sequence. KorB_{pFBAOT6} is most closely related to KorB of the related environmental plasmids pIPO2 (59) and pSB102 (52). It does not harbor a putative helix-turn-helix motif required for DNA binding (but does possess a ParB-like nuclease domain), although some of the conserved residues in this region are present (33). The absence of *O_B* in pFBAOT6 and no obvious means of KorB-DNA interaction mean that the role of KorB_{pFBAOT6} in transcription repression is not obvious and may be less encompassing than the role in IncP plasmids. Its role in binding IncC2 may also be affected as in IncP KorB the IncC interaction domain overlaps the H-T-H region (Fig. 3)

FIG. 3. Multiple-sequence alignment of KorB_{pFBAOT6} with its closest relatives from plasmids pIPO2 and pSB102 and the IncP representative R751. A black background indicates amino acids that are fully conserved, while a gray background indicates amino acids that are 50% conserved. The black and gray bars below the sequence of R751 indicate the helix-turn-helix motif and the IncC interaction domain, respectively. The repeating 18- or 19-amino-acid sequence in KorB_{pFBAOT6} is enclosed in a box and is labeled I to VI.

(32). Another feature of KorB_{pFBAOT6} is that it is considerably larger than KorB of IncP plasmids (452 aa as opposed to 350 to 360 aa). In a way similar to the pFBAOT6 *kfrA* gene, this is due to the presence of six 57-bp tandem repeats that translate into to a repeating 19-amino-acid sequence in the third and most variable (33) quarter of the protein (Fig. 3).

pFBAOT6.08 is a *korA/trfB* homologue that encodes a protein with a putative H-T-H motif. However, as with O_B, pFBAOT6 does not appear to carry the KorA operator (O_A) consensus sequence (GTTTAGCTAAAC) that is conserved in IncP-1 plasmids RK2 and R751 (26), and so its role in transcription repression is unclear. pFBAOT6.09 encodes a putative IncC2 homologue with an ATPase motif that is characteristic of ParA proteins. In IncP plasmids this motif is required for symmetric distribution of KorB-DNA complexes (36, 59).

oriT and DNA transfer region (Dtr). A putative *oriT* sequence has been identified in pFBAOT6 (Fig. 4). It carries a *nic* site which is common to IncP-1 plasmids (61) and to a trio of related environmental plasmids, pXF51, pIPO2, and pSB102 (59), but which in pFBAOT6 differs in the first base of the sequence by alteration of a Y (generally a T) to an A. The proposed *oriT* is also similar to those of the plasmids mentioned above in that it is located in an intergenic region and contains several inverted repeats that may be important for target site recognition during DNA processing (Fig. 4). As in *oriT* in pXF51, pIPO2, and pSB102, the *nic* site is located on the reverse complementary strand, suggesting a common feature that the transfer genes are transferred last (59). The region lies upstream of two putative mobilization genes, pFBAOT6.13 and pFBAOT6.14. The predicted product of pFBAOT6.13 (MobC) is most closely related to that of *orf21* (from pIPO2) and to MobA (from pXF51). The pFBAOT6.14

predicted protein is related to the VirD2 homologue nickases TraR and MobB (from pIPO2 and pXF51, respectively). pFBAOT6 MobC probably shares the predicted *nic* site recognition role with MobA in pXF51 (35). pFBAOT6 VirD2 contains the three highly conserved relaxase domains that are common to nickases and relaxases of IncP plasmids, of plasmid Ti, and of plasmids pXF51, pIPO2, and pSB102 (42, 59). Nickases are responsible for forming a DNA-protein complex that is translocated to the recipient cell during conjugation by nicking the DNA at a specific target site. Plasmids pFBAOT6, pXF51, pIPO2, and pSB102 also have a common distinguishing feature in that the relaxase gene is transcribed toward the other Dtr genes. Immediately downstream from the two mobilization genes are three genes (pFBAOT6.15, pFBAOT6.15A, and pFBAOT6.16) which correspond to IncP genes *traC3*, *traD*, and *traC4*. These genes encode putative primases that probably act together to form the primosome. The *traC4* gene homologue contains a 39-bp sequence that is repeated 5.7 times in the middle of the gene. pFBAOT6.30 (*top*) encodes a putative topoisomerase I which is most closely related to topoisomerases of pXF51 and pIPO2 (57 and 47% identity, respectively). As in pXF51, pIPO2, and pSB102, the topoisomerase gene is separated from the other Dtr genes by the genes encoding the mating pair formation (Mpf) complex. Its location in these three plasmids between genes encoding a putative lytic transglycosylase (a VirB1-like protein) and the major prepilin subunit is one of four diagnostic features that define a new group of environmental BHR plasmids (52, 59). In each of these plasmids the *virB1* homologue stop codon is <10 nucleotides from the start codon of the topoisomerase, which is indicative of an operon. In pFBAOT6 the start codon of the topoisomerase gene overlaps the stop codon of pFBAOT6.31. The predicted product of pFBAOT6.31 possesses an amino-ter-

FIG. 4. Proposed *oriT* region of pFBAOT6 aligned with *oriT* regions from pXF51, pIPO2, and pSB102. The nucleotide numbers for the region in pFBAOT6 are indicated on the left. A black background indicates amino acids that are fully conserved, while a gray background indicates amino acids that are 50% conserved. Inverted repeat sequences are shown only for pFBAOT6 and are indicated by arrows above the sequence and numbers corresponding to the repeats. The *nic* site is indicated by a solid triangle above the sequence.

minimal signal peptide sequence and is most closely related to a putative protein from *Sinorhizobium meliloti* phage PCB5 and to an amidase from *C. perfringens* strain E88. In *Bacillus subtilis* lytic transglycosylases act on the glycan backbone, while amidases are peptidoglycan hydrolases that act on the *N*-acetylmuramoyl-L-alanine side chain of the glycan backbone (55). VirB1-like proteins and lytic transglycosylases have a common motif and are implicated in causing localized lysis of the peptidoglycan layer during pilus assembly (11). pFBAOT6 does not possess a *virB1* gene homologue, but, if it is an amidase, the product of pFBAOT6.31 may have a function analogous to that of VirB1. It is also possible that the product of pFBAOT6.33 (a putative exported protein), which carries a signal sequence and three probable transmembrane helices, acts in conjunction with pFBAOT6.31. These two reverse complement strand genes are separated by an IS630 family insertion (IS) element (see below).

Mating pair formation. Conjugation involves the transfer of DNA across the cell envelopes of donor and recipient cells. In IncP plasmids such as RP4, it is proposed that mating pair formation and relaxosome complexes, connected by a TraG-like protein (such as TraG or VirD4), facilitate this process (61). In RP4 the Mpf complex is encoded in an operon which is analogous to the *virB* operon in *Agrobacterium tumefaciens* (11). Both systems operate as type IV secretion pathways and harbor genes in a colinear arrangement suggestive of a common ancestral origin (11). In pFBAOT6, genes proposed to be involved in mating pair formation are located in a region spanning approximately 12 kb (pFBAOT6.17 to pFBAOT6.29) and encode putative proteins that most closely resemble those of the *virB* system.

The region begins with CDSs pFBAOT6.25 to pFBAOT6.28, which appear to constitute a putative operon (the greatest distance between start and stop codons is 18 nucleotides) and are

homologues of *virB2* to *virB5*. pFBAOT6.28 encodes a VirB2-like protein which is the major pilus subunit (10, 11) and has a signal sequence and three probable transmembrane helices. VirB3 (pFBAOT6.27) and VirB5 (pFBAOT6.26) are thought to be minor pilus subunits, while VirB4 (pFBAOT6.25) possesses a Walker A nucleotide-binding motif (P-loop) and therefore possibly ATPase activity and is likely to be involved in transport at the cytoplasmic membrane (11). A VirB6-like protein which probably spans the cytoplasmic membrane and acts as a channel former is encoded by pFBAOT6.23. To this end, pFBAOT6 VirB6 contains a signal peptide sequence, a membrane lipoprotein lipid attachment site, and an additional six predicted transmembrane helices. In *A. tumefaciens* VirB7 is a lipoprotein that interacts with VirB9 and the T pilus (5, 14, 15, 48), a function analogous to that of TraH in RP4 (61). In pFBAOT6, pFBAOT6.22, which is situated between the *virB6* and *virB8* homologues, encodes a putative lipoprotein. This protein is 68 aa long (VirB7 is 47 to 69 aa long [10]), and the highest level of similarity (35% identity) is with the amino-terminal region of the product of *Brucella suis* ORF12, which is also part of a *virB* operon (40). Similarly, the product of ORF65 of plasmid pXF51 is a VirB7 homologue which resides between ORF11 (a VirB6 homologue) and ORF12 (a VirB8 homologue) and which exhibits 48% identity over the first 34 amino acid residues with ORF12 of *B. suis* (35). The products of both pXF51 ORF65 and pFBAOT6.22 contain amino-terminal signal sites for protein cleavage. Considering its position in the operon and the other similarities that it exhibits with other VirB7 homologues, the product of pFBAOT6.22 is likely to have a VirB7-like role. Proteins VirB8, VirB9, and VirB10 (predicted products of pFBAOT6.19 to pFBAOT6.21) are thought to interact with each other to form a transport pore (13), while VirB11 (pFBAOT6.18) is a TraG-like ATPase with a Walker A nucleotide-binding motif that is thought to operate in the cytoplasm for

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.1                               60.                               120.
ctaaatagctgttccgaatagtagatcacccgaggaaggaactcagtcagttgtgcgactgattagtcgtaacaaacaagccacaccaaggaactgagttgagcctatcct
                                                                                               M P I L
                                                                                               180.                               240.
accacctctgccacgaccacaacggcgccgcatcacaatcatccatgccaccggtgataaaggacatgctcgtcgttgatggccatcttctgtttacatgaaggacgcacgatcac
P P L P R P Q R R R I H K I I H A T R D K G H A R R L M A I L L L H E G R T I T
                                                                                               300.                               360.
cgatgttcatcacctaccggcgctgcgcgctcgaccatcggtcgttggtcgttcgagatgaaggcatagatgcgctagaagccttacggctggccgctgcccagctcttacc
D V H H L T G A A R S T I G R W L R W Y R D E G I D A L E A L P A G R A P V L P
                                                                                               420.                               480.
tategccaagattgtcaccttactggtgctgctcatgcagttttctccgcaagattttggttatcagcgcagccgttggtgtactgaactattggctataaaaatcaatcggttaacagg
I A K I V T L L V L L M Q F S P Q D F G Y Q R S R W C T E L L A I K I N R L T G
                                                                                               540.                               600.
gctcaatgtggcagcctccacgctccgcgcatggttacctcgatggcattgtctggcgaggcccggtgccgaccttgccgataaaagaccagcctatcaggaaaaatggcgaaat
L N V A A S T L R R W L P R M G I V W R R P V P T L R I K D P A Y Q E K M A N I
                                                                                               660.                               720.
cgacgcgcgctggccgctgtgatgccataatccggtgtttatgagcgatgaagtcgatataagactcaacccaattaggagccgactggatgttcagagcgcagcaaaagcggtt
D A A L A R C D A D N P V F Y E D E V D I D L N P K L G A D W M F R A Q Q K R V
                                                                                               780.                               840.
tctcacaccggggcagaatgccagcattatctggcagcgcttctccacgcaggcaacgggaaggggtactttacgtcagcgggatcaagaaaaactcatcgctgtttattgccatgctgga
V T P G Q N A K H Y L A G V L H A G N G R V L Y V S G I K K N S S L F I A M L E
                                                                                               900.                               960.
aaagctgcgcaccactaccgacgagccaaaaccatcagcttgatcctcgataactacatcattcacaaaagcaaacagaccgagcgatggttgaagaagaatcccaagttctgctggt
K L R H H Y R R A K T I T L I L D N Y I I H K S K Q T E R W L K K N P K F C L V
                                                                                               1020.                               1080.
gttcagccagtttacagcccttgggttaatcacatagaacggctatggcacaagttacatgaaccatcacgcgcaatcatcaatgcagagggatggcaaacctgctggcccgagtgaa
F Q P V Y S P W V N H I E R L W H K L H E T I T R N H Q C R G M A N L L A R V K
                                                                                               1140.                               1164
acattcatggataccgctgtctcttcccggggaatggctatggcacagcgaaggtgtagcactattgggatcagttatttag
H F M D T V S P F P G N G Y G T A K V *

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FIG. 5. Translated nucleotide sequence of the IS630 family transposon found in pFBAOT6. Inverted repeat ends are indicated by italics. The underlined region is the H-T-H motif. The conserved residues of the DDE motif of the transposase are indicated by larger type.

type IV secretion pathway complex assembly (44). At the end of this cluster is a VirD4 homologue (pFBAOT6.17) which also has a function analogous to that of TraG. Although it bears no Walker A motif that is characteristic of TraG proteins, it has three probable transmembrane helices which are essential in transferred-DNA transport from *A. tumefaciens* to the plant cell (31).

Interestingly, the *virB* homologue operon in pFBAOT6 is interrupted at two points by genes that are not obviously related to the operon (pFBAOT6.24 and pFBAOT6.29). In both cases the encoded proteins are related to proteins encoded in a similar region in plasmids pXF51 and pIPO2 (35, 59). pFBAOT6.24 divides the *virB5* and *virB6* homologues and encodes a putative lipoprotein consisting of 89 amino acids. The closest relative of this protein is TraG (not the same TraG involved in coupling reactions) in pIPO2 (39% identity), whose gene is located in the same position (between the *virB5* and *virB6* homologues *traF* and *traH*) in the plasmid. In turn, the closest relative of this protein is a hypothetical protein from pXF51 whose gene is situated (and duplicated) between *virB5* and *virB6* homologues. pFBAOT6.29 separates the *top* gene from the prepilin gene *virB2*. The protein encoded by pFBAOT6.29 is most similar (51% identity) to a protein with an unknown function encoded by ORF4 in pXF51.

The genetic load of pFBAOT6. The core functions of pFBAOT6 are encoded by genes in the first 31 to 32 kb of the plasmid. The remaining 53 to 54 kb is occupied by accessory genes which constitute the genetic load of the plasmid (50). Genes found in this highly mosaic region are nearly entirely accounted for by a class I integron (occupying approximately 8.5 kb) and what appears to be a 43-kb composite transposon. The only region of significance outside this integron and transposon is occupied by an IS630 family IS element.

IS630 family IS element. The IS630 family IS element in pFBAOT6 lies between pFBAOT6.31 and pFBAOT6.33 at positions 30507 to 31670. This element is 1,164 bp long and contains imperfect 22-bp IR ends (Fig. 5). The predicted transposase (TnpA) is most similar to transposases from the IS630 family IS element of *Shewanella oediensis* and from an IS630 relative found on a novel integrative and conjugative element in *E. coli* (53). Each of these transposases possesses the D(79)D(35)E motif (shown for pFBAOT6 in Fig. 5). No obvious target site duplication that could signify a simple transposition event was found at the target site. However, this is typical of IS630 family transposition as target sites are often difficult to discern from IRs (34).

In4-like integron. The region between positions 33478 and 40444 contains genes and features of a class I integron (Fig. 6). This integron is similar to that found in the pFBAOT6-related IncU plasmid pRAS1 (57). Both integrons share features with integron In4, especially in having IS6100 after *orf6*. However, In4 contains both complete and partial copies of IS6100, while in both pRAS1 and pFBAOT6 only the complete copy is present. The integrons of pRAS1 and pFBAOT6 are different in that pRAS1 has the *dfrA16* cassette (trimethoprim resistance) and pFBAOT6 carries the streptomycin resistance cassette *aadA2*. This finding accounts for the two resistance phenotypes of the plasmids (45, 49). In addition, pRAS1 contains a sequence (466 nucleotides) downstream of the IS6100 *mpA* gene that encodes a truncated resolvase (57). pFBAOT6 contains a complete version of the *res* gene (encoding a 189-aa protein) that over the shared length is identical to that in pRAS1. It also encodes a second resolvase having a separate origin at the more customary left end of the integron (Fig. 6). This is preceded by pFBAOT6.47, which encodes a putative

FIG. 6. Comparison of class I integrons contained in pFBAOT6 and the related IncU plasmid pRAS1. The In4-like region shared by the two plasmids is almost identical except for the presence of the *dfrA16* cassette in pRAS1 instead of the *aadA2* gene in pFBAOT6. The integron in pFBAOT6 is flanked by complete *res* genes (having separate origins), and *res* is identical over the shared length to the truncated *resA* gene of pRAS1.

conserved hypothetical protein that is 71% identical to hypothetical protein Xf2080 encoded by IncP-1 β multiresistance plasmid pB10 (50). Upstream of this gene is a third inverted repeat sequence, IRL.

43-kb Tn1721-based transposon. Plasmid pFBAOT6 was originally described as a member of a group of IncU plasmids that have a common backbone (45). Outside the common backbone pFBAOT6 differs from the related IncU plasmids due to the presence of a considerable amount of extra DNA (45). This extra DNA appears to account for approximately 43 kb, and this region may have been acquired in a single transposition event. The first description of pFBAOT6 and its relatives was based on the presence of the TetA tetracycline resistance determinant in a variant of Tn1721 (45). Tn1721 is a member of the Tn501 subfamily of Tn3 family transposons. It comprises Tn1722 at the left end (consisting of *orfI*, which encodes a methyl-accepting chemotaxis protein [MCP]), *tnpR* (resolvase) and *tnpA* (transposase) genes, a *res* site, and inverted repeats IRL and IRR I, which collectively permit Tn1722 to transpose independent of the right half of Tn1721. The right half of Tn1721 contains the relaxase gene *tccA*, a truncated *tnpA* gene (*tnpA'*), a *pecM*-like gene (50), *tet* genes *tetA(A)* and *tetR(A)*, and IRR II (2). Based on the fact that the DNA probe derived from the MCP region hybridized to a 9-kb EcoRI fragment rather than the expected 5.5-kb fragment (45), it was proposed that pFBAOT6 contains a complete Tn1721 which has an altered IRL region (which contains an EcoRI site). However, this study showed that the two halves of Tn1721 (designated Tn1721-L and Tn1721-R) (Fig. 1) are separated. Tn1721-L contains inverted repeats IRL and IRR I and should be independently transposable. However, Tn1721-R is flanked by direct repeats of IRR which should prevent transposition independent of Tn1721-L. Collectively, the area flanked by Tn1721-L and Tn1721-R spans a 43-kb region. The two extreme IRs (IRL at position 41003 and IRR at position 83801) are flanked by 5-bp (TGTTG) direct repeats indicative of a simple Tn1721 transposition event (18). This suggests that Tn1721-L and Tn1721-R may have transferred the intervening genes as a 43-kb transposon. Another possibility is that a smaller Tn1721-based transposon originally transposed and has been added to by subsequent recombination and transposition events.

IS4 family insertion sequence flanks a composite transposon. pFBAOT6 Tn1721-L contains complete *orfI* (MCP) and *tnpR* genes, a *res* site, and a truncated *tnpA* gene (designated *tnpAΔ1*). The truncation is due to insertion of a composite transposon carrying a previously undescribed IS element at each end (Fig. 1 and 7). Insertion of this transposon cleaved the gene at the 3'-OH end, resulting in a 963-aa transposase (TnpAΔ1) instead of the usual 988-aa transposase. The remaining region of the interrupted gene lies inside IRR I and probably remains untranscribed. It is not known whether TnpAΔ1 is functional, although the seven residues which are totally conserved in Tn3 family transposons (the last six of which are presumed to be in the active site) are present in TnpAΔ1 (based on Tn3 numbering these residues are D₂₉₄, D₆₈₉, D₇₆₅, N₈₉₂, E₈₉₅, N₉₃₈, and P₉₆₆ [18]). Transposition into the transposase gene generated an 8-bp duplication (5'-ACC GGCGA-3'). The flanking IS element ends are each approximately 1,390 bp long (1,391 bp for IS_L and 1,386 bp for IS_R), exhibit 67% identity with each other, and have imperfect 20-mer inverted repeat ends (Fig. 7). The *tnpA* genes which have the highest levels of identity to the *tnpA* genes of IS_L and IS_R are in ISSB1 in plasmid p37 from the marine psychrophilic bacterium Mst37 (accession number AJ305328) and, to a lesser extent, in ISH8 (and isoelements ISH8A to ISH8E) found in plasmid pNRC100 in *Halobacterium* sp. strain NRC-1 (accession number AF016485). Each of the TnpA proteins belongs to the IS4 family, whose members carry the highly conserved DDE motif that is also present in other IS families (such as IS3 and IS6) and in retroviral integrases (34). This triad motif is involved in catalysis by presumably coordinating divalent metal cations (in particular, Mg²⁺) in order to assist nucleophilic attacks during transposition (34). Alignment of the transposase from this element with its closest relatives highlighted the DDE motif, as well as several other conserved regions (Fig. 7). The composite transposon contains pFBAOT6.52, which encodes a 123-aa predicted protein which exhibits no homology with any other protein in the database.

Tn1721-R is located at position 78234 and begins with another 15-aa part of TnpA that corresponds to residues 974 to 988 of the 988-aa protein. This is followed by IRR, *tccA*, the tetracycline resistance genes *tetR(A)* and *tet(A)*, a *pecM*-like gene, the common Tn1721 truncated transposase gene (*tnpA'*),

A

B

TnpA (IS _L)	LAGFEQVLLQDGSSSFALHPQLA- (68) -LNNKLLADAGYIS- (114) -LYRCRWQIELLFKELKS
TnpA (IS _R)	LGAFKQVLLHDGTSFAVHRRLA- (68) -LTNCLLLADAGYID- (114) -LYRCRWQVELLFKEWKS
ISSB1	LSAFKGIVLQDGSSFAVHDSLK- (68) -LEGRLLADRGYFK- (113) -LYQLRWQIELLFKEWKS
ISH8	IDRFRDVMADGTVLRLEHFLS- (76) -LHGRLILLDLAYFK- (126) -LYRCRWEVETLFRELKT
ISH8A	IDRFRDVMADGTVLRLEHFLS- (76) -LENRLVLFDLAYFK- (126) -LYRCRWEVELLFRELKT
Consens.	F D H L L D Y Y W E L E K

FIG. 7. Organization of the composite transposon that is inserted into Tn1721-L. (A) The composite transposon is flanked by IS4 family IS elements (IS_L and IS_R) that contain the inverted repeats IR1 to IR4. The green region represents CDS pFBAOT6.52. (B) Regions of the transposase proteins of IS_L and IS_R aligned with the regions that exhibit the highest levels of identity, highlighting the DDE domain. The numbers in parentheses indicate the numbers of amino acids between the two conserved D residues and between the second conserved D residue and the conserved E residue (circled in the consensus [Consens.] sequence). ISSB1 is from plasmid p37 in marine psychrophilic bacterium Mst37 (accession number AJ305328); ISH8 and ISH8A are found in *Halobacterium* sp. strain NRC-1 plasmid pNRC100 (accession number AF016485).

and IRR. The 47-bp region that forms part of the Tn1721 *tnpA* gene may be a relic of a previous recombination event.

Tn3 family transposon. A Tn3 family transposon is present immediately upstream of Tn1721-R. This transposon occupies a 6.5-kb region between positions 71780 and 78234 and is flanked by typical 38-bp inverted repeat ends. It contains a putative *tnpA* gene and eight other putative genes. Its location in pFBAOT6 has not generated a target site duplication, and so it is unlikely to have been acquired by simple transposition unless the transposition was followed by one or more deletion events. The transposase (TnpA) is expected to be functional as it is 988 aa long (like Tn3 family transposases) and exhibits 92.3% identity at the amino acid level with TnpA from Tn21. Tn3 family transposons typically encode a site-specific recombinase (TnpR) and have a *res* site to which TnpR binds during cointegrate resolution (18). The Tn3-like transposon does not have an obvious *tnpR* homologue and therefore may not be able to resolve cointegrates. However, the right half of the transposon contains an *invA* gene (pFBAOT6.89), which encodes a putative DNA invertase that contains a resolvase-like H-T-H domain. This might functionally replace the missing resolvase. Also in the right half is a cluster of genes (pFBAOT6.82 to pFBAOT6.89) that are virtually identical to the genes found in the *D. acidovorans* IncP-1 β plasmid pUO1 (pUO1 ORF6 to ORF10 and *invA* [58]). This region may have been acquired by homologous recombination from a pUO1 relative. pUO1 also contains the mating pair formation genes *trbA* to *trbP* between transposons TnHad2 and Tn4672 (58). CDSs pFBAOT6.78 to pFBAOT6.80 located outside the left

IR of the Tn3 family transposon in pFBAOT6 are most similar to the mating pair formation genes *trbI*, *trbG*, and *trbF* (partial, interrupted by the downstream Tn3 family transposon), and therefore, based on their proximity to the transposon, it is possible that they were acquired from a pUO1-like plasmid.

The remaining CDSs in the 43 kb transposon (pFBAOT6.54 to pFBAOT6.77) form a mosaic collection of 24 genes that exhibit the highest levels of similarity to chromosomal genes of several plant-associated bacteria, such as *Pseudomonas syringae*, *Xanthomonas campestris*, *Sinorhizobium meliloti*, *Bradyrhizobium japonicum*, and *Mesorhizobium loti*. These genes occur in clusters of two or three genes and align with other genes generally associated with gene regulation and stress responses. These genes include the genes that encode LysR family transcriptional regulators (pFBAOT6.67 and pFBAOT6.77), MarR family transcriptional regulators (pFBAOT6.71 and pFBAOT6.76), peptide methionine sulfoxide reductases (pFBAOT6.65, pFBAOT6.66, and pFBAOT6.69), glutathione S-transferase (pFBAOT6.64), zinc-binding dehydrogenase (pFBAOT6.70), and the endoribonuclease L-PSP family protein (pFBAOT6.60). Also in this region are nine CDSs (pFBAOT6.55, pFBAOT6.57, pFBAOT6.58, pFBAOT6.61, pFBAOT6.62, pFBAOT6.68, pFBAOT6.72, pFBAOT6.74 and pFBAOT6.75) that encode hypothetical proteins with unspecified functions.

Concluding remarks. This is the first description of the complete sequence of an IncU plasmid. Based on observations made by several groups (1, 45, 57), IncU plasmids appear to have a common backbone and differ in a variable region con-

taining resistance-determining genes. This report describes sequence analyses of the core regions (replication, maintenance, and transfer) that make up this common backbone. Based on this sequence and restriction fragment length polymorphism observations made in other studies, these essential features are unlikely to differ greatly in the related IncU plasmids. However, pFBAOT6 differs from other reported IncU plasmids by being almost twice as large due to the presence of 43 kb that may have been acquired in a single transposition event. This region includes many genes that appear to exhibit the highest levels of identity with genes of plant-associated bacteria. The common backbone is very similar to those of plasmids pXF51, pIPO2, and pSB102 found in plant-associated bacterial hosts. These plasmids have been shown to constitute a new group of environmentally important BHR plasmids (52, 59), based on the following criteria (52): (i) the presence of a putative topoisomerase gene between Mpf genes coding for a putative lytic transglycosylase and a putative prepilin; (ii) the orientation of the relaxase gene, which is opposite that of the Dtr genes; (iii) an *ssb* gene preceding similarly organized maintenance genes; and (iv) the presence of at least one pair of large unrelated direct repeats spanning several hundred base pairs. Plasmid pFBAOT6 has some but not all of these features. The presence of a gene encoding a putative amidase upstream of the topoisomerase gene and the *traC/virB2* homologue is analogous to criterion i. The amidase differs from a transglycosylase in cleaving side chains of the peptidoglycan backbone and not the backbone itself (55), but its role is analogous to that of VirB1-like transglycosylases in that it likely facilitates DNA-protein transport by localized digestion of the peptidoglycan layer. The *mobC* and *virD2* genes have an orientation opposite that of the Dtr genes and therefore satisfy criterion ii. Criterion iii was not fulfilled as the order of the genes (*korC-traN-kfiA-korA-incC-korB*, compared to *ssb-incC-korB-unknown-korA*) more closely resembled the order in IncP plasmids, such as RP4. An obvious *ssb* gene homologue has not been found in pFBAOT6, and *incC2* is instead transcribed directly after *korA*, which differs from what occurs in pXF51, pIPO2, and pSB102. Similarly, pFBAOT6 does not satisfy criterion iv as it does not possess pairs of large unrelated direct repeats that span several hundred base pairs. The repeats that flank the *rep* gene are not considered to qualify in the same way as the direct repeats in the plasmids mentioned above. Since two of the four criteria are fulfilled, it appears that the structural organization of the backbone of pFBAOT6 represents a link between the structural organization of the backbone of IncP plasmids and the structural organization of the backbone of the three environmental BHR plasmids. Whether pFBAOT6 should be included in the same group with the environmental BHR plasmids is debatable as it may represent an evolutionary midpoint since its origin was not a plant-associated bacterial host but a hospital effluent *A. caviae* strain. This bacterium, like IncU plasmids, is globally ubiquitous and has been found in a wide range of environments, including the rhizospheres of plants (22, 23), the larva of the silkworm (22, 23, 28), and the common housefly, which can act as a vector for transfer of the bacterium to human food items (37, 38). It was proposed previously that pIPO2, pXF51, and pSB102 might be variants of an archetypal plasmid class associated with phytosphere bacteria (37, 38, 59). The sequence of events that led to the *A. caviae* strain exam-

ined harboring a multiresistance plasmid whose backbone organization is very closely related to that of plasmids in plant-associated bacteria is unknown. However, the presence of the plasmid in this strain suggests that the environmental family of BHR plasmids should not be thought of as a family that is exclusive to plant-associated bacteria or to any one type of environment.

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