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Complete Sequence of p07-406, a 24,179-Base-Pair Plasmid Harboring the bla_{VIM-7} Metallo-β-Lactamase Gene in a *Pseudomonas aeruginosa* Isolate from the United States▽

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An outbreak involving a *Pseudomonas aeruginosa* strain that was resistant to all tested antimicrobials except polymyxin B occurred in a hospital in Houston, TX. Previous studies on this strain showed that it possesses a novel mobile metallo-β-lactamase (MBL) gene, designated bla_{VIM-7}, located on a plasmid (p07-406). Here, we report the complete sequence, annotation, and functional characterization of this plasmid. p07-406 is 24,179 bp in length, and 29 open reading frames were identified related to known or putatively recognized proteins. Analysis of this plasmid showed it to be comprised of four distinct regions: (i) a region of 5,200 bp having a Tn*501*-like mercuric resistance (*mer*) transposon upstream of the replication region; (ii) a Tn*3*-like transposon carrying a truncated integron with a bla_{VIM-7} gene and an insertion sequence inserted at the other end of this transposon; (iii) a region of four genes, upstream of the Tn*3*-like transposon, possessing very high similarity to plasmid pXcB from *Xanthomonas campestris* pv. *citri* commonly associated with plants; (iv) a backbone sequence similar to the backbone structure of the IncP group plasmid Rms149, pB10, and R751. This is the first plasmid to be sequenced carrying an MBL gene and highlights the amelioration of DNA segments from disparate origins, most noticeably from plant pathogens.

In recent years reports of clinical isolates of *Pseudomonas aeruginosa* resistant to all β-lactams have become increasingly common. In some geographical regions this resistance has risen to approximately 40% to all antipseudomonal β-lactams, including carbapenems (1, 40). A number of these isolates have been shown to produce a metallo-β-lactamase (MBL) enzyme encoded by the transferable gene bla_{IMP}, bla_{VIM}, bla_{SPM}, or $bla_{GIM.}$ (28, 39).

The VIM MBL family was first described in 1999 from a *P. aeruginosa* isolate in Italy. Subsequently, it was found in different species in Europe, Asia, and, more recently, in the United States (5, 7, 13, 14, 17, 19, 21, 22, 23–25, 27, 33). To date, more than 20 different VIM-type enzymes (http://www.lahey.org) have been identified; the dominant type is VIM-2, which has been found in more than 30 countries (3, 10, 14, 20, 21, 23, 26, 27, 30, 31, 39, 40). The bla_{VIM} gene is often carried on mobile gene cassettes inserted into class 1 integrons and is located either chromosomally or carried on plasmids.

P. aeruginosa strain 07-406 was isolated at a hospital in Houston, TX, from sputum of a cancer patient who presented with pneumonia. This isolate was resistant to all antimicrobials except polymyxin B, according to standard testing methods (4), and also gave a positive result with the MBL Etest strip (AB Biodisk, Solna, Sweden) (37).

The MBL gene ($bla_{VIM-7)}$ from *P. aeruginosa* 07-406 and its immediate genetic context have been previously characterized (37). The encoded enzyme shares 77% identity with VIM-1 and 74% with VIM-2; it is the most divergent of the VIM MBLs characterized thus far and constitutes the third subgroup among the VIM-type β-lactamase family. bla_{VIM-7} was shown to be located on a plasmid of approximately 24 kb (37).

Herein, we report the full nucleotide sequence of plasmid p07-406, including its complete annotation, and examine its functional characteristics.

MATERIALS AND METHODS

Bacterial strains and plasmids. *P. aeruginosa* strain 07-406 carrying plasmid p07-406 was used for plasmid isolation. *Escherichia coli* strain DH5α [λ^- ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 recA1 endA1 hsdR17*(r_K^- m_K^-) *supE44 thi-1 gyrA relA1*] was used as the recipient host for p07-406, and its fragments were cloned into pK18 as described previously (38).

Antimicrobials, reagents, and mercury susceptibility testing. Antimicrobial agents used in this study were ceftazidime (GlaxoSmithKline, Worthing, United Kingdom) and kanamycin (Sigma Chemical Co., St. Louis, MO). Other general reagents were purchased from Sigma Chemical Co. or BDH (Poole, United Kingdom). Mercury resistance testing was carried out by plating 10^5 CFU in 5 μl onto Muller Hinton agar containing serial dilutions of mercury (HgCl) in a manner similar to one previously described (18).

Plasmid subcloning construction, sequencing, and sequence analysis. p07-406 plasmid DNA was isolated by an alkaline lysis method and the Qiagen maxi plasmid isolation kit (Qiagen Ltd., Cranley, United Kingdom). Plasmid DNA was restricted into four fragments (7.3 kb, 6.3 kb, 5.5 kb, and 5 kb) by EcoRI, which were subcloned into pK18 (38). Clones were sequenced on both strands by the dideoxynucleotide chain termination method. (ABC Sequencing Centre, Imperial College, London, United Kingdom). Sequence reads were assembled by Seqman (DNASTAR software [http://www.ebi.ac.uk/fasta33/nucleotide.html]), and finishing methods were included using the parent plasmid as a sequencing template for completing sequences across each junction. Sequencing analysis was performed with the Lasergene DNASTAR software package. The nucleotides were searched for potential open reading frames (ORFs) by using BLAST (http://www.ebi.ac.uk/blast2) against the EMBL prokaryotic database (http://www.ebi.ac.uk/embl/index.html).

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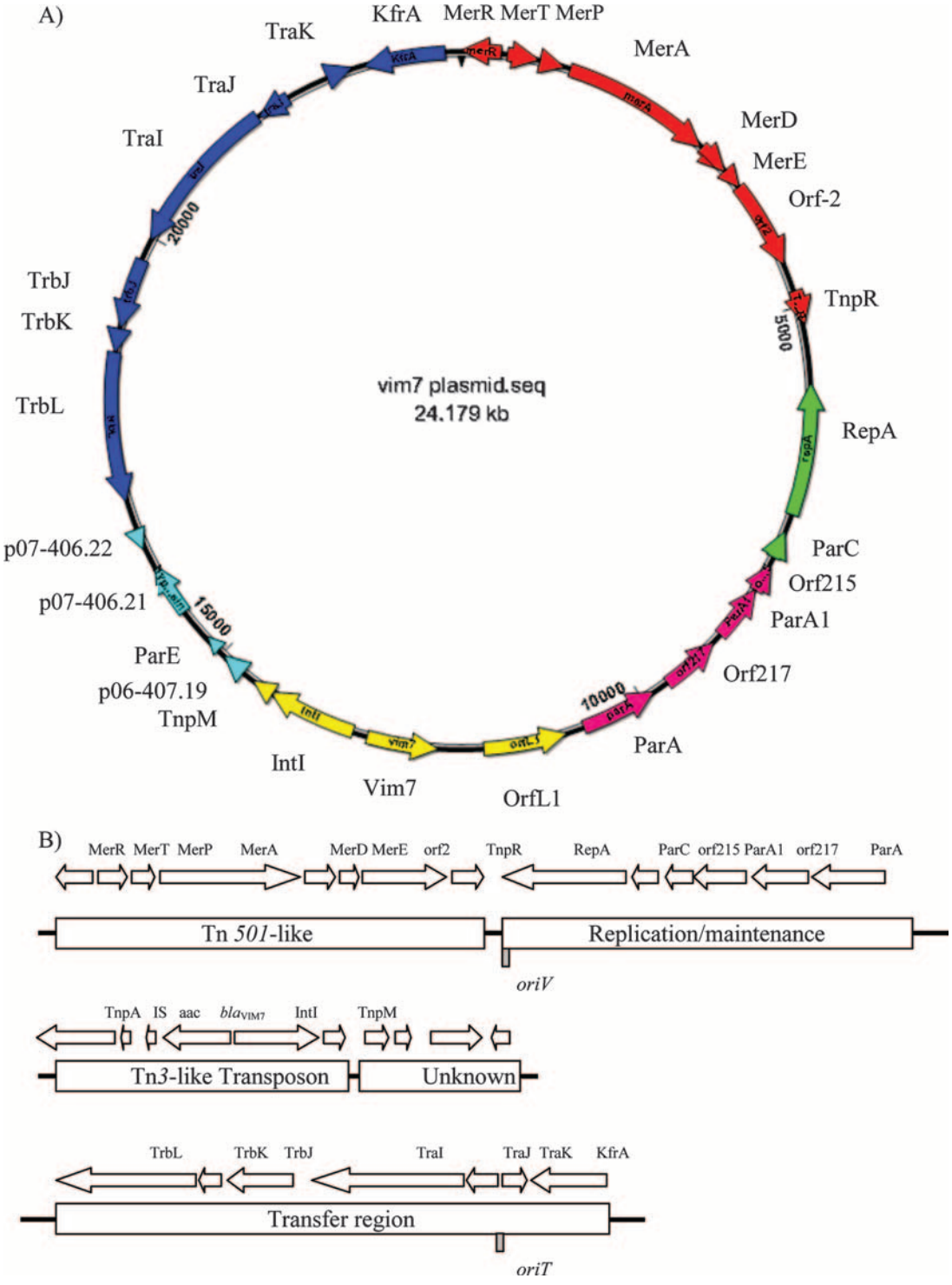

FIG. 1. (A) Genetic map of plasmid p07-406 (accession number AM778842) showing the arrangement of the major DNA segments. Blue, Tra region; red, Mer; green, Rep; pink, ParA and ParC; yellow, class 1 integron containing bla_{VIM-7}; and aqua, ParE. (B) Physical maps of the regions containing transposons and transfer regions in plasmid p07-406.

Conjugations. Donor (strain 07-406) and recipient bacterial cells (rifampin-resistant mutants of *E. coli* DH5α and *P. aeruginosa* strain PAO1) were grown separately to mid-log phase and harvested by centrifugation (at 12,000 × *g*), and the supernatant was discarded. The pellets were resuspended, and the cell suspensions were mixed together in a donor/recipient ratio of 1:1, spread on a nutrient agar plate without selective antibiotics, and incubated for 18 h at 37°C. The cell mixture was then plated onto selective medium (ceftazidime at 10 and 50 μg/ml and rifampin at 50 μg/ml) and incubated for 18 h at 37°C. *E. coli* DH5α and *P. aeruginosa* strain PAO1 carrying pUB6061 (kanamycin resistance) were used as positive controls for the mating experiments, as previously described (2).

Electroporation of p07-406. Plasmid p07-406 was extracted using a Qiagen maxi kit and transformed by electroporation into rifampin-resistant mutants of

TABLE 1. Predicted ORFs in plasmid p07-406

Name	Position (bp)	No. of amino acids	Direction of transcription[a]	% GC content	Amino acid identity to informative database match		Reference or accession no.
					% Identity	Description of homolog (aa position)[b]	
MerR	1–435	144	←	61.38	95.8	Mercuric resistance operon regulatory protein from Tn*501* of *P. aeruginosa* plasmid pVS1 (1–144)	Z00027
MerT	507–857	116	→	61.82	93.1	Mercuric transport protein from Tn*501* of *P. aeruginosa* plasmid pVS1	Z00027
MerP	873–1148	91	→	60.51	94.5	Mercuric transport periplasmic protein from Tn*501* of *P. aeruginosa* plasmid pVS1	Z00027
MerA	1220–2905	561	→	65.72	99.8	Mercuric reductase from Tn*501* of *P. aeruginosa* plasmid pVS1	Z00027
MerD	2923–3288	121	→	69.67	99	HTH-type transcriptional regulator *mer*D (mercuric resistance protein *mer*D) from Tn*501* of *P. aeruginosa* plasmid pVS1	Z00027
MerE	3285–3521	78	→	64.56	100	Hypothetical mercuric resistance protein from Tn*501* of *P. aeruginosa* plasmid pVS1	Z00027
Orf2	3518–4507	329	→	62.22	99.6	Orf2 from Tn*501* of *P. aeruginosa* plasmid pVS1 (157–485, equivalent to aa 1–329)	Z00027
TnpR	4823–5200	125	→	60.05	88.4	Transposition resolvase from Tn*501* of *P. aeruginosa* plasmid pVS1	Z00027
RepA	5853–7328	491	←	66.73	75.3	Replication protein from *P. aeruginosa* plasmid Rms149	NC007100
ParC	7527–7841	104	←	60.95	44.9	Putative partitioning-associated protein from plasmid Rms149 (6–92)	NC007100
Orf215	7925–8254	109	←	66.36	94.5	Hypothetical protein Orf215 from *X. campestris* pv. *citri* plasmid pXcB (1–109)	AY228335
ParA1	8247–8879	210	←	66.82	100	ParA1 from *X. campestris* pv. *citri* plasmid pXcB (1–210)	AY228335
Orf217	8993–9664	223	←	61.31	94	Hypothetical protein Orf217 from *X. campestris* pv. *citri* plasmid pXcB (3–223, equivalent to 44–264)	AY228335
ParA	9847–10758	303	←	68.2	84.5	Resolvase ParA *X. axonopodis* pv. *citri* (17–300)	XAC3375
p07-406.15	10913–11845	310	←	61.64	76	Transposase OrfL1 from *Janthinobacterium* spp.	AB095952
					68	Putative transposase TnpA from *A. avenae* subsp. *citrulli*	
Vim7	12348–13145	265	←	54.51	100	bla_{VIM-7}	37
IntI	13311–14324	337	→	61.14	100	Integrase/recombinase IntI-1 of *P. aeruginosa*	37
TnpM	14293–14550	85	→	60.47	98.7	Transposase TnpM	NC004989
p07-406.19	14690–14971	93	→	67.02	75.5	Hypothetical protein pB8_006 encoded by plasmid pB8	AJ863570
					43.9	YacA of *X. campestris* pv. *citri* plasmid pXcB	
p07-406.20	15058–15249	63	→	64.06	80	Plasmid stabilization protein ParE from plasmid pB8	NC007502
					52	YacB of *X. campestris* pv. *citri* plasmid pXcB	AY228325
p07-406.21	15626–16234	202	→	67.32	74.6	Hypothetical protein Paer03001361 from *P. aeruginosa* UCBPP-PA14 (123–200)	AY257539
					75.5	Hypothetical protein TNCP25 (36–80)	
p07-406.22	16449–16682	77	←	64.96	57	Hypothetical protein BPSL3261 from *Burkholderia pseudomallei* (1–75)	NC007502
TrbL	17006–18667	553	←	65.46	70	COG3846; type IV secretory pathway, TrbL components from *P. aeruginosa* (1–546)	AJ564903
TrbK	18664–18930	88	←	64.42	54	Entry exclusion protein	AJ564903
TrbJ	18946–19731	261	←	64.5	76	Mating pair formation protein (1–256)	AJ564903
					75	Conjugal transfer/entry exclusion protein precursor (1–256)	
TraI	19971–21779	602	←	70.87	69.1	DNA relaxase (1–546)	AJ564903
TraJ	21797–22174	125	←	67.99	73.9	Conjugal transfer *oriT*-binding protein	AJ564903
TraK	22657–22956	99	→	64.33	49.5	Conjugal transfer *oriT*-binding protein TraK	AJ564903
KfrA	23091–23996	301	←	71.41	55.6	KfrA transcriptional regulator protein	AJ877225

[a] In accordance with Fig. 1: ←, leading strand; →, complementary DNA strand.
[b] aa, amino acids (aa position refers to the encoding sequence of that particular DNA structure); HTH, helix-turn-helix; *A. avenae*, *Acidovorax avenae*; *B. pseudomallei*, *Burkholderia pseudomallei*.

either *E. coli* DH5α or *P. aeruginosa* PAO1. Electroporation was carried out at 2.5 kV, 25 μF, and 200 Ω with a Genepulser apparatus (Bio-Rad Laboratories, Corston, United Kingdom). Electrotransformants were selected on LB medium supplemented with ceftazidime (10 and 50 μg/ml) and rifampin (50 μg/ml).

Nucleotide sequence accession number. The full sequence of plasmid p07-406 has been deposited under accession number AM 778842.

RESULTS AND DISCUSSION

General features of p07-406. Plasmid p07-406 consists 24,179 nucleotides and possesses an overall GC content of 63.81%. It is predicted to contain a total of 29 ORFs possessing significant

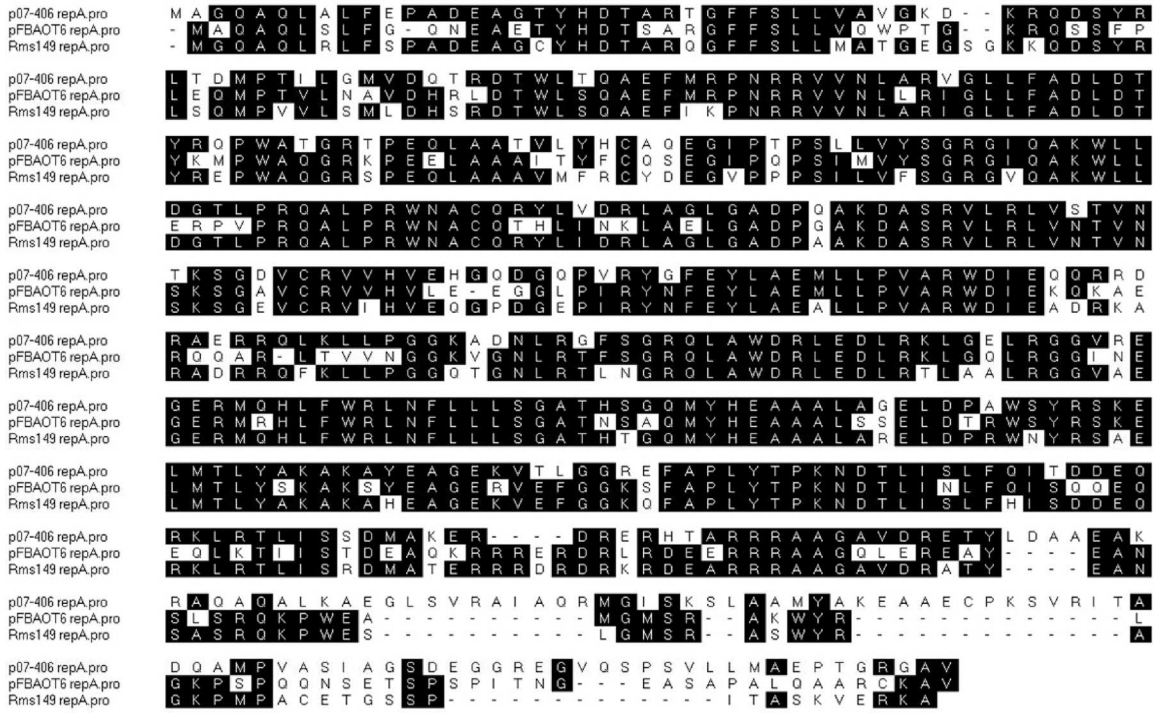

FIG. 2. Alignment of replication protein (RepA pro) of p07-406 with its closest relatives from IncP-6 plasmid Rms 149 and IncU plasmid pFBAOT6 (accession numbers NC007100 and CR376602, respectively). A black background indicates amino acids that are fully conserved.

homology to functional proteins from databases. The deduced physical and genetic maps of p07-406 are shown in Fig. 1, and the genes are listed in Table 1. Numbering of this plasmid starts with the *merR* gene in the Tn*501*-like transposon. The main regions in this plasmid are the following: (i) a region of 5,200 bp having a Tn*501*-like mercuric resistance (*mer*) transposon upstream of replication region; (ii) a Tn*3*-like transposon carrying a truncated integron with the bla_{VIM-7} gene and an insertion sequence inserted at the other end of this transposon; (iii) a region of four genes, upstream of the Tn*3*-like transposon, possessing very high similarity to a plasmid pXcB from *Xanthomonas campestris* pv. *citri* commonly associated with plants; and (iv) a backbone sequence similar to the backbone structure of the IncP group plasmids Rms149, pB10, and R751.

Replication and partition region. The putative gene for replication (*rep*) of p07-406 was identified and was found to encode a 491-amino-acid protein. The highest level of similarity at the amino acid level is 75.3% identity to the putative replication protein from the IncP-6 plasmid, Rms149, characterized from *P. aeruginosa* strain Ps142 (accession no. NC007100). This Rep protein is also related (68% identity) to the replication protein encoded by an IncU plasmid, pFBAOT6 (accession no. CR376602), characterized from *Aeromonas caviae* (Fig. 2). The replicative origins of these two plasmids remain unclear (9, 29). Furthermore, we were unable to identify any of the promoter consensus sequences in the *rep* gene (36). Most notably, the series of identical direct repeat sequences proposed to be involved in the replication function in both Rms149 and pFBAOT6 (two copies in Rms 149 and five copies in pFBAOT6; accession numbers NC007100 and CR376602, respectively) were not found in the corresponding region of plasmid p07-406. Therefore, the *rep* region carried by p07-406 is clearly atypical.

The *parC* gene immediately next to the *rep* gene is most closely related to the *parC* gene (44.9%) carried on the IncP-6 plasmid Rms149 (accession no. NC007100). Interestingly, a cluster of three genes downstream of *parC* show very high identity (94.5%, 100%, and 94%) to *orf215*, *parA1*, and *orf217*, respectively, carried on plasmid pXcB from a *X. campestris* pv. *citri* strain, originating from South America and involved in the disease citrus canker (accession numbers NC005240 and AY228335).

The last gene of this region was predicted to encode a resolvase protein possessing 84.5% identity to ParA in *Xanthomonas axonopodis* pv. *citri* (accession number NC003921). There is a 16-bp inverted repeat flanking the *orf215* and *parA1* genes and a predicted stem-loop structure between the *orf217* and *parA* genes. The *parABC* genes play a very important role in the inheritance of plasmid (6, 12, 34, 35); however, thus far, no definitive function has been attributed to either *orf215* or *orf217*. It is unclear as to whether p07-406 possesses a ParB-type function and, if so, which ORF serves this role. It is possible that this is in part addressed by the function of KfrA.

In p07-406 the *kfrA* gene is separated from this section by insertion of a Tn*501*-like transposon between *kfrA* and *repA*. The predicted product of *kfrA* possesses 57% identity to the KfrA protein from plasmids R751 or pADP-1 (accession numbers AJ877225 and NC004956, respectively).

E. coli and the *P. aeruginosa* PAO1 strain were transformed to ceftazidime resistance by p07-406. The transformants appeared to be very stable, with 100% retention after 20 passages

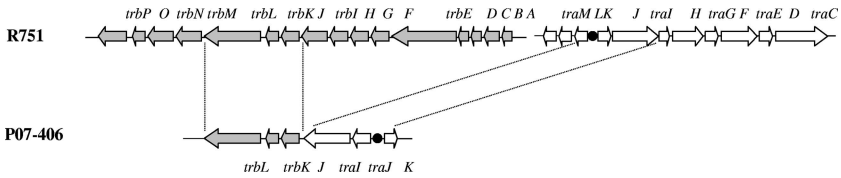

FIG. 3. Comparison of the transfer regions of plasmids p07-406 and R751. Genes are shown by arrows indicating the direction of transcription. The *trb* region is represented by gray fill; the *tra* region is shown as open arrows. The origin of transfer (*oriT*) in R751 and the predicted *oriT* are denoted by black circles. Genes encoding similar products are connected by broken lines. In the case of R751, the *tra* and *trb* regions are not adjacent to each other.

in nonselective growth medium; accordingly, it may be assumed that the *kfrA* gene from p07-406 is functional.

Nonfunctional transfer region. To determine the transfer region of p07-406, conjugation experiments were undertaken to examine the possibility of transferring the plasmid from *P. aeruginosa* 07-406 (donor) to *E. coli* DH5α and *P. aeruginosa* PAO1 (recipients). p07-406 failed to transfer to the recipient strains using conjugation under laboratory conditions. However, p07-406 could be readily transferred by electroporation into both *E. coli* DH5α and *P. aeruginosa* PAO1.

The proposed conjugative region between bp 17006 and 22956 possesses a GC content of 66.3% and contains the loci *trb* (*trbL*, *trbK*, and *trbJ*) and *tra* (*traI*, *traJ*, and *traK*). These regions are very closely related to their corresponding counterparts of the IncP group plasmid pB10 (accession number AJ564903) isolated from a wastewater treatment plant and another IncP group plasmid pADP-1 (accession number NC 004956), as well as R751 (accession number AJ877225). The transfer region of p07-406 is approximately 6 kb. Comparisons with the functional transfer region of related plasmid R751 (Fig. 3) showed that there are many more genes present and involved in its conjugative transfer function, although in R751 the *tra* and *trb* regions are interrupted by insertion of a transposable element (accession numbers X5548 and U07618). The essential *tra* function genes *traF* and *traG* are missing in p07-406 (16). The *trb* region possesses genes (*trbM*, *trbN*, and *trbP*) which are known not to be essential for plasmid transfer and all are missing from p07-406 (14, 15). The largest segment normally associated with this locus and present in R751, *trbA* to *trbI* (accession number U07618) which is thought to play an unknown role in transfer, is also absent in p07-406.

We attempted to identify the putative *oriT* of p07-406 by comparing it to the corresponding *oriT* sequence from plasmids R751 and RP4 (Fig. 4). The predicted *oriT* sequences are highly conserved and contain several inverted repeats that may be involved in target recognition during DNA processing. These data also suggest that the lack of transfer functions possessed by p07-406 may not relate to the *oriT* structure. These data would explain the lack of conjugation under experimental conditions.

Plasmid p07-406 contains a Tn*501*-like transposon. p07-406 possesses a 5,200-bp region encoding the mercuric resistance (*mer*) transposon which has a GC content of 63% (Table 1). This mercuric resistance region of p07-406 is highly homologous to the transposon Tn*501* even though it does not possess the *tnpA* gene required for transposition (accession number Z00027). The *merA* region of p07-406 displays 99.8% identity to the *merA* gene in Tn*501*. Adjacent to this region is the helix-turn-helix-type transcriptional regulator gene *mer*D and another mercuric resistance gene, *merE*. Downstream of *merD* and *merE* is a large ORF, *orf2*, encoding a protein of 329 amino acids which is thought to play a role in the signaling cascade to MerR and MerD. This internal resolution site (*res*) located upstream of the *tnpR* gene is 127 bp long and homologous to the *res* site in Tn*501*. The transposase gene, *tnpA*, commonly found in Tn*501* is not present in p07-406, presumably because it was deleted during the transposition event into this plasmid. In p07-406, there is a 25-bp inverted repeat sequence CGTG CTTTATTTTCCGTTTTCTGAG/CTCAGAAAACGGAAA ATAAAGCACG immediately flanking the Tn*501*-like transposon.

The level or resistance to Hg ions was 8 μg/ml in *E. coli* carrying p07-406 and 16 μg/ml in the host strain (*P. aeruginosa* 07-406) compared to 0.25 μg/ml for the *E. coli* DH5α alone, indicating that the *mer* region is likely to be functional.

The Tn*3* family transposon carries a truncated integron with the bla_{VIM-7} gene and an insertion sequence. There is a second transposition element present in this plasmid between bp 10913 and 14550 immediately downstream of the partition region and possessing a GC content of 59%. This region has a truncated class 1 integron, carrying the bla_{VIM-7} gene, inserted into transposase gene *tnpM.* This gene is also truncated, only having 257 bp left from the N terminus, but the amino acid sequence showed 98% identity to other transposases from var-

FIG. 4. Proposed *oriT* region of p07-406 aligned with the *oriT* region from plasmids R751 and RP4. The nucleotide numbers for the region in p07-406 are indicated on the right. A black background indicates residues that are fully conserved. seq, sequence.

ious plasmids including R478 and pAPEC-O1-R (8, 11). Another transposase gene, *orfL1*, possessing 70% identity to an insertion element from *Janthinobacterium* spp., is located downstream of the *aacA4*. Therefore, given the evidence, it is likely that *orfL1* inserted into the *aac* gene cassette of the class 1 integron (also carrying bla_{VIM-7}) and that this region inserted into transposase gene *tnpM*.

Other unknown regions. p07-406 possesses a large section of DNA (bp 14690 to 16682, between the Tn*3*-like transposon and the conjugative transfer region) having a GC content of 66%, but the encoded products from these ORFs have no defined function. Genes denoted p07-406.19 and p07-406.20 (downstream of the Tn*3*-like transposon) show homology to genes carried on the plasmid, pB8, from *P. aeruginosa.* p07-406.19 displays 75% identity to a unknown gene, *orf3*, and p07-406.20 encodes a protein possessing 80% identity to a plasmid stabilization protein, ParE (encoded on plasmid pB8) (32). Genes p07-406.21 and p07-406.22 (adjacent to the aforementioned p07-406.19 and p07-406.20) also encode proteins of undefined function. However, it is possible that this region is involved in either plasmid stabilization and/or transfer.

Conclusions. Our molecular studies on plasmid p07-406 have revealed significant sections of the plasmid containing DNA related to the plant pathogen *X. campestris* pv. *citri.* The overall GC content of p07-406 is 64%, which suggests that it did not originate in *Enterobacteriaceae* but in environmental bacteria such as *Pseudomonas* or *Xanthomonas* (36). The two main segments of p07-406 (for conjugative transfer and mating-pair formation, on the one hand, and replication and stable inheritance, on the other) are derived from different ancestral IncP-type plasmids. Different sections of this plasmid suggest that p07-406 has been created from both environmental (plants) and clinical DNA segments. This is the first report of the complete nucleotide sequence of a plasmid harboring an MBL gene, in this case, bla_{VIM-7}, from the United States.

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REFERENCES

1. **Andrade, S. S., R. N. Jones, A. C. Gales, and H. S. Sader.** 2003. Increasing prevalence of antimicrobial resistance among *Pseudomonas aeruginosa* isolates in Latin American medical centres: 5 year report of the SENTRY Antimicrobial Surveillance Program (1997–2001). J. Antimicrob. Chemother. **52:**140–147.
2. **Avison, M. B., T. R. Walsh, and P. M. Bennett.** 2001. pUb6060: a broad-host-range, DNA polymerase-I-independent ColE2-like plasmid. Plasmid **45:**88–100.
3. **Cardoso, O., R. Leitao, A. Figueiredo, J. C. Sousa, A. Duarte, and L. V. Peixe.** 2002. Metallo-β-lactamase VIM-2 in clinical isolates of *Pseudomonas aeruginosa* from Portugal. Microb. Drug. Resist. **8:**93–97.
4. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing, 15th informational supplement. CLSI/NCCLS M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Cornaglia, G., A. Mazzariol, L. Lauretti, G. M. Rossolini, and R. Fontana.** 2000. Hospital outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-1, a novel transferable metallo-β-lactamase. Clin. Infect. Dis. **31:**1119–1125.
6. **Gerlitz, M., O. Hrabak, and H. Schwab.** 1990. Partitioning of broad-host-range plasmid RP4 is a complex system involving site-specific recombination. J. Bacteriol. **172:**6194–6203.
7. **Giakkoupi, P., A. Xanthaki, M. Kanelopoulou, A. Vlahaki, V. Miriagou, S. Kontou, E. Papafraggas, H. Malamou-Lada, L. S. Tzouvelekis, N. J. Legakis, and A. C. Vatopoulos.** 2003. VIM-1 metallo-β-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. J. Clin. Microbiol. **41:**3893–3896.
8. **Gilmour, M. W., N. R. Thomson, M. Sanders, J. Parkhill, and D. E. Taylor.** 2004. The complete nucleotide sequence of the resistance plasmid R478: defining the backbone components of incompatibility group H conjugative plasmids through comparative genomics. Plasmid **52:**182–202.
9. **Haines, A. S., K. Jones, M. Cheung, and C. M. Thomas.** 2005. The IncP-6 plasmid Rms149 consists of a small mobilizable backbone with multiple large insertions. J. Bacteriol. **187:**4728–4738.
10. **Jeong, S. H., K. Lee, Y. Chong, J. H. Yum, S. H. Lee, H. J. Choi, J. M. Kim, K. H. Park, B. H. Han, S. W. Lee, and T. S. Jeong.** 2003. Characterization of a new integron containing VIM-2, a metallo-β-lactamase gene cassette, in a clinical isolate of *Enterobacter cloacae*. J. Antimicrob. Chemother. **51:**397–400.
11. **Johnson, T. J., Y. M. Wannemeuhler, and J. A. Scaccianoce.** 2006. Complete DNA sequence, comparative genomics, and prevalence of an IncHI2 plasmid occurring among extraintestinal pathogenic *Escherichia coli* isolates. Antimicrob. Agents Chemother. **50:**3929–3933.
12. **Kwong, S. M., C. C. Yeo, and C. L. Poh.** 2001. Molecular analysis of the pRA2 partitioning region: ParB autoregulates *parAB* transcription and forms a nucleoprotein complex with the plasmid partition site, *parS*. Mol. Microbiol. **40:**621–633.
13. **Lauretti, L., M. L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G. M. Rossolini.** 1999. Cloning and characterization of bla_{VIM}, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents Chemother. **43:**1548–1590.
14. **Lee, K., J. B. Lim, J. H. Yum, D. Yong, Y. Chong, J. M. Kim, and D. M. Livermore.** 2002. bla_{VIM-2} cassette-containing novel integrons in metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. Antimicrob. Agents and Chemother. **46:**1053–1058.
15. **Lessl, M., D. Balzer, K. Weyrauch, and E. Lanka.** 1993. The mating pair formation system of plasmid RP4 defined by RSF1010 mobilization and donor-specific phage propagation. J. Bacteriol. **175:**6415–6425.
16. **Lessl, M., W. Pansegrau, and E. Lanka.** 1992. Relationship of DNA-transfer-systems: essential transfer factors of plasmids RP4, Ti and F share common sequences. Nucleic Acids Res. **25:**6099–6100.
17. **Lombardi, G., F. Luzzaro, J. D. Docquier, M. L. Riccio, M. Perilli, A. Coli, G. Amicosante, G. M. Rossolini, and A. Toniolo.** 2002. Nosocomial infections caused by multidrug-resistant isolates of *Pseudomonas putida* producing VIM-1 metallo-β-lactamase. J. Clin. Microbiol. **40:**4051–4055.
18. **Mantengoli, E., and G. M. Rossolini.** 2005. Tn*5393*d, a complex Tn*5393* derivative carrying the PER-1 extended-spectrum β-lactamase gene and other resistance determinants. Antimicrob. Agents Chemother. **49:**3289–3296.
19. **Mavroidi, A., A. Tsakris, E. Tzelepi, S. Pournaras, V. Loukova, and L. S. Tzouvelekis.** 2000. Carbapenem-hydrolysing VIM-2 metallo-β-lactamase in *Pseudomonas aeruginosa* from Greece. J. Antimicrob. Chemother. **46:**1041–1042.
20. **Mendes, R. E., M. Castanheira, P. Garcia, M. Guzman, M. A. Toleman, T. R. Walsh, and R. N. Jones.** 2004. First isolation of bla_{VIM-2} in Latin America: report from the SENTRY Antimicrobial Surveillance Program. Antimicrob. Agents Chemother. **48:**1433–1434.
21. **Pallecchi, L., M. L. Riccio, J. D. Docquier, R. Fontana, and G. M. Rossolini.** 2001. Molecular heterogeneity of bla_{VIM-2}-containing integrons from *Pseudomonas aeruginosa* plasmids encoding the VIM-2 metallo-β-lactamase. FEMS Microbiol. Lett. **195:**45–50.
22. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J. D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
23. **Poirel, L., L. Collet, and P. Nordmann.** 2000. Carbapenem-hydrolyzing metallo-β-lactamase from a nosocomial isolate of *Pseudomonas aeruginosa* in France. Emerg. Infect. Dis. **6:**84–85.
24. **Poirel, L., T. Lambert, S. Turkoglu, E. Ramo, L. Gaillard, and P. Nordmann.** 2001. Characterization of class 1 integrons from *Pseudomonas aeruginosa* that contain the bla_{VIM-2} carbapenem-hydrolyzing β-lactamase gene and of two novel aminoglycoside resistance gene cassettes. Antimicrob. Agents Chemother. **45:**546–552.
25. **Pournaras, S., A. Tsakris, M. Maniati, L. S. Tzouvelekis, and A. N. Maniatis.** 2002. Novel variant (bla_{VIM-4}) of the metallo-β-lactamase gene bla_{VIM-1} in a clinical strain of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **46:**4026–4028.
26. **Pournaras, S., M. Maniati, E. Petinaki, L. S. Tzouvelekis, A. Tsakris, N. J. Legakis, and A. N. Maniatis.** 2003. Hospital outbreak of multiple clones of *Pseudomonas aeruginosa* carrying the unrelated metallo-β-lactamase gene variants bla_{VIM-2} and bla_{VIM-4}. J. Antimicrob. Chemother. **51:**1409–1414.
27. **Prats, G., E. Miro, B. Mirelis, L. Poirel S. Bellais, and P. Nordmann.** 2002. First isolation of a carbapenem-hydrolyzing β-lactamase in *Pseudomonas aeruginosa* in Spain. Antimicrob. Agents Chemother. **46:**932–933.
28. **Queenan, A. M., and K. Bush.** 2007. Carbapenemases: the versatile β-lactamases. Clin. Microbiol. Rev. **20:**440–458.
29. **Rhodes, G., J. Parkhill, C. Bird, K. Ambrose, M. C. Jones, G. Huys, J.**

Swings, and R. W. Pickup. 2004. Complete nucleotide sequence of the conjugative tetracycline resistance plasmid pFBAOT6, a member of a group of IncU plasmid with global ubiquity. Appl. Environ. Microbiol. **70:**7497–7510.

30. **Riccio, M. L., L. Pallecchi, R. Fontana, and G. M. Rossolini.** 2001. In*70* of plasmid pAX22, a *bla*$_{VIM-1}$-containing integron carrying a new aminoglycoside phosphotransferase gene cassette. Antimicrob. Agents Chemother. **45:** 1249–1253.
31. **Sardelic, S., L. Pallecchi, V. Punda-Polic, and G. M. Rossolini.** 2003. Carbapenem-resistant *Pseudomonas aeruginosa*-carrying VIM-2 metallo-β-lactamase determinants, Croatia. Emerg. Infect. Dis. **9:**1022–1023.
32. **Schluter, A., H. Heuer, R. Szczepanowski, S. M. Poler, S. Schneiker, A. Puhler, and E. M. Top.** 2005. Plasmid pB8 is closely related to the prototype IncP-1 β plasmid R751 but transfers poorly to *Escherichia coli* and carries a new transposon encoding a small multi-drug resistance efflux protein. Plasmid **54:**135–148.
33. **Scoulica, E. V., I. K. Neonakis, A. I. Gikas, and Y. J. Tselentis.** 2004. Spread of *bla*$_{VIM-1}$-producing *Escherichia coli* in a university hospital in Greece. Genetic analysis of the integron carrying the *bla*$_{VIM-1}$ metallo-β-lactamase gene. Diagn. Microbiol. Infect. Dis. **48:**167–172.
34. **Sia, E. A., R. C. Roberts, C. Easter, D. R. Helinski, and D. H. Figurski.** 1995. Different relative importance of the par operons and the effect of conjugal transfer on the maintenance of intact promiscuous plasmid RK2. J. Bacteriol. **177:**2789–2797.
35. **Sobecky, P. A., C. L. Easter, P. D. Bear, and D. R. Helinski.** 1996. Characterization of the stable maintenance properties of the par region of broad-host-range plasmid RK2. J. Bacteriol. **178:**2086–2093.
36. **Thorsted, P. B., D. P. Macartney, P. Akhtar, A. S. Haines, N. Ali, P. Davidson, T. Stafford, M. J. Pocklington, W. Pansegrau, B. M. Wilkins, E. Lanka, and C. M. Thomas.** 1998. Complete sequence of the IncP beta plasmid R751: implications for evolution and organisation of the IncP backbone. J. Mol. Biol. **282:**969–990.
37. **Toleman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh.** 2004. *bla*$_{VIM-7}$, an evolutionarily distinct metallo-β-lactamase gene in a *Pseudomonas aeruginosa* isolate from the United States. Antimicrob. Agents Chemother. **48:** 329–332.
38. **Walsh, T. R., M. A. Toleman, W. Hryniewicz, P. M. Bennett, and R. N. Jones.** 2003. Evolution of an integron carrying *bla*$_{VIM-2}$ in Eastern Europe: report from the SENTRY Antimicrobial Surveillance Program. J. Antimicrob. Chemother. **52:**116–119.
39. **Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann.** 2005. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325.
40. **Zavascki, A. P., P. B. Gaspareto, A. F. Martins, A. L. Goncalves, and A. L. Barth.** 2005. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing SPM-1 metallo-β-lactamase in a teaching hospital in southern Brazil. J. Antimicrob. Chemother. **56:**1148–1151.