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Functional Analysis of Insertion Sequence IS*Aba1*, Responsible for Genomic Plasticity of *Acinetobacter baumannii*▽

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IS*Aba1* is an insertion sequence that is widely distributed in *Acinetobacter baumannii*. We demonstrated here that IS*Aba1* and the composite transposon Tn*2006* are capable of transposition, generating 9-bp target site duplications. The expression of the IS*Aba1* transposase-encoding gene was downregulated by translational frameshifting.

Insertion sequences (IS) are the smallest and the most abundant transposable elements (<2.5 kb) capable of independent transposition in microbial genomes (20). They cause insertion mutations and genome rearrangements and enhance the spread of resistance and virulence determinants within species (2, 12, 16, 18, 23). Based on previous in silico analyses, IS*Aba1* is bracketed by 15-bp short inverted repeat sequences. IS*Aba1* is bound by 9-bp short direct repeats that correspond to target site duplications likely generated upon transposition and possesses the acidic amino acid triad DDE (5, 10, 13).

IS*Aba1* has been identified in *Acinetobacter baumannii* (10), which is a gram-negative bacterium causing nosocomial outbreaks and showing a multidrug resistance phenotype (28, 34). IS*Aba1* has been identified in association with several antibiotic resistance genes in *A. baumannii* (17, 26, 30, 32). The role of IS*Aba1* in the expression of the antibiotic resistance gene of *A. baumannii* has been demonstrated for bla_{ampC}, encoding the naturally occurring cephalosporinase, and for the $bla_{\text{OXA-23}}$ gene, encoding a carbapenem-hydrolyzing oxacillinase, but it might act similarly with other resistance genes (6, 7, 10). IS*Aba1* might also be responsible for the mobility of $bla_{\text{OXA-23}}$, with two copies bracketing this ß-lactamase gene and forming a composite transposon (defined as Tn*2006*) (7). The main objective of this study was to determine the functionality of IS*Aba1* as a mobile element and to analyze its impact on the plasticity of the *A. baumannii* genome.

In order to follow the transposition of IS*Aba1*, this element was tagged. An ScaI restriction site was inserted upstream of the transposase coding sequence and downstream of the right inverted repeat in order to not impair the transposase-encoding gene, using primers pre-IS*Aba1*-5′ext and IS*Aba1*-3′extScaI (Table 1). IS*Aba1*-ScaI was cloned in pCR-BluntII-TOPO vector (kanamycin resistant). A PCR product corresponding to the entire $bla_{\text{TEM-1}}$ gene sequence, encoding an ampicillin resistance marker (25), was inserted in the ScaI restriction site, giving rise to recombinant plasmid pIS*Aba1*-TEM-1. The recombinant plasmid, introduced in the *Escherichia coli* TOP10 recipient strain by electrotransformation as previously described (21), was selected on Trypticase soy agar plates containing kanamycin (30 μg/ml) and amoxicillin (50 μg/ml). The recombinant plasmid pIS*Aba1*-TEM-1 was electroporated into *E. coli* RZ211 (pOX38-Gen; Gen^r, a transfer-proficient F plasmid derivative) for transposition experiments (8). Selection was performed on gentamicin (8 μg/ml)-, kanamycin (30 μg/ml)-, and amoxicillin (50 μg/ml)-containing plates. *E. coli* RZ211 was then used as a donor for mating-out assays with *E. coli* J53 Azide^r, with selection on gentamicin (8 μg/ml) and azide (100 μg/ml) with or without amoxicillin (50 μg/ml)-containing plates, as described previously (14, 22). The transposition frequency was calculated by dividing the number of Gen^r Amx^r Azide^r transconjugants by the number of Gen^r Azide^r transconjugants. All of the Gen^r Amx^r Azide^r colonies were screened for kanamycin susceptibility to exclude those that may have resulted from nontransposition events. The transposition frequency (mean plus or minus standard deviation) determined in *E. coli* (IS*Aba1*-TEM-1) was $(2.1 \pm 0.7) \times 10^{-7}$. (For the measurement of transposition frequencies, standard deviation was calculated from three independent cultures. Statistical analysis was performed using the Student *t* test; a *P* value of ≤0.05 was considered significant.)

The insertion sites of IS*Aba1*-TEM-1 were determined with seven randomly chosen Gen^r Amx^r Azide^r Kan^s transconjugants by DNA sequencing (29) the external neighboring regions of the inverted repeats using primers IS*Aba1*-5′ext and IS*Aba1*-3′ext (Applied Biosystems 3100 sequencer) (Table 1). A 9-bp target site duplication, consistent with a transposition event, was observed. Insertions had occurred on seven different sites, and alignment of those insertion site sequences together with those identified in the genome of *A. baumannii* AYE, a multidrug-resistant clinical isolate (24, 33), revealed a consensus motif (AAATAAATT) (see Fig. 2) corresponding to an AT-rich target site sequence.

Since changes in growth conditions may affect the transposition efficiency of several mobile elements (19), the transposition of IS*Aba1* was determined after addition of several antibiotics as described previously (14). The antibiotic concentrations studied were 1/2, 1/4, and 1/10 of the MICs. The ciprofloxacin effect was studied since fluoroquinolones have

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TABLE 1. Sequences of primers used in this study

Primer[a]	Sequence (5′→3′)	Reference or source
IS*Aba1*a (+)	ATGCAGCGCTTCTTTGCAGG	9
IS*Aba1*b (−)	AATGATTGGTGACAATGAAG	9
Pre-IS*Aba1*5′ext (+)	GCATGATTAGCTCCTCTG	This work
IS*Aba1*3′extScaI (−)	ATCCTCTGTACACGACAAATATACTTCACAGAACCC	This work
IS*Aba1*-GSP1 (−)	TGAAAACATATTGAAAATCA	This work
IS*Aba1*-GSP2 (−)	GAAGCGCTGCATACGTCGAT	This work
IS*Aba1*-GSP3 (−)	GTGGTAAGCACTTGATGGGC	This work
IS*Aba1*mut1 (+)	AACTTCTGCAATCGTGTTAAAAAGAACTTCATTGTCACC	This work
IS*Aba1*mut2 (−)	GGTGACAATGAAGTTCTTTTTAACACGAATGC	This work
Pre-TEM1 (+)	GTATCCGCTCATGAGACAATA	23
Pre-TEM2 (−)	TCTAAAGTATATATGAGTAAACTTGGTCTG	23
IS*Aba1*5′ext (+)	CTCTACACATATCATAAGGCAGC	This work
Pre-TEM3′ext (−)	CCTACTTGCTTTATCCTGTCTAGCCG	This work
T3	AATTAACCCTCACTAAAGGG	Stratagene
T7	CGGGATATCACTCAGCATAATG	Stratagene
UAP	CTACTACTACTAGGCAGGCGTCGACTAGTAC	Invitrogen

[a] +, forward; −, reverse.

been shown to induce antibiotic resistance in *Vibrio cholerae* through an SOS-mediated response (3). Transposition assays were performed as described above. No statistical difference was observed for IS*Aba1* transposition frequency by adding different antibiotics at the studied concentrations (Table 2).

Translational control of transposition by frameshifting has been demonstrated for several IS (9, 15). IS*Aba1* contains two consecutive and overlapping open reading frames (ORFs), with the second ORF (*orfB*) at phase −1 relative to the upstream ORF (*orfA*) at phase 0 (Fig. 1). It was therefore likely that a frameshift might be necessary to give rise to a unique and likely functional transposase (5, 27). Analysis of the IS*Aba1* sequence showed a frameshift motif, made of seven adenosines at position 458. The AAA AAA A motif was changed to AAA AA<u>G</u> AA in order to generate a coding sequence that does not require any frameshifting and therefore to generate a constitutive expression of the transposase gene (the change is underlined). Site-directed mutagenesis was performed to add a guanine residue on the pIS*Aba1*-TEM-1 plasmid, yielding the pIS*Aba1*-mut-TEM-1 plasmid, according to the manufacturer's recommendations (QuikChange site-directed mutagenesis kit; Stratagene). Transposition experiments were performed as described above. Transposition efficiency (transposition frequency mean plus or minus standard deviation) was $(1.8 \pm 5.0) \times 10^{-4}$ using the modified IS*Aba1* (IS*Aba1*-mut-TEM-1), corresponding to a 1,000-fold increase compared to that of the native IS*Aba1* (Tn*2006*; see below). This increased transpo-

TABLE 2. IS*Aba1* transposition frequency in *E. coli* without and with antibiotics[a]

Antibiotic (μg/ml)	Transposition frequency [(mean ± SD) × 10^{-7}]
None	2.1 ± 1.0
Ceftazidime (0.12)	1.8 ± 0.8
Ceftazidime (0.06)	2.4 ± 0.5
Ceftazidime (0.02)	7.5 ± 5.8
Cefotaxime (0.02)	5.2 ± 1.7
Cefotaxime (0.01)	3.2 ± 2.1
Cefotaxime (0.005)	4.0 ± 3.2
Ciprofloxacin (0.001)	3.3 ± 2.6
Ciprofloxacin (0.0005)	3.6 ± 0.6
Ciprofloxacin (0.0002)	2.6 ± 1.0
Imipenem (0.06)	1.4 ± 1.1
Imipenem (0.03)	6.6 ± 6.1
Imipenem (0.01)	3.4 ± 2.0
Nalidixic acid (0.5)	4.5 ± 3.1
Nalidixic acid (0.25)	1.2 ± 0.7
Nalidixic acid (0.1)	1.6 ± 1.0
Piperacillin (6)	2.9 ± 0.2
Piperacillin (3)	1.8 ± 0.2
Piperacillin (1)	3.1 ± 3.8

[a] In each case, three independent experiments were performed, and the mean and standard deviations were calculated. Statistical analysis was performed using the Student *t* test, and a *P* value of ≤0.05 was considered significant. MICs for the *E. coli* RZ211 strain (pIS*Aba1*-TEM-1) were as follows: ceftazidime, 0.25 μg/ml; cefotaxime, 0.047 μg/ml; ciprofloxacin, 0.002 μg/ml; imipenem, 0.12 μg/ml; nalidixic acid, 1 μg/ml; and piperacillin, 12 μg/ml.

TABLE 3. Distribution of IS*Aba1* in *Acinetobacter* spp.

Species	Place(s) of origin	No. of isolate(s)	No. of positive isolate(s)[b]
Acinetobacter baumannii	Various[a]	50	40
Acinetobacter haemolyticus	Belgium	2	2
Acinetobacter johnsonii	France	3	1
Acinetobacter junii	France	9	7
Acinetobacter lwoffii	France	3	2
	Germany	1	1
Acinetobacter radioresistens	France	3	0
Acinetobacter schindleri	France	1	0
Acinetobacter ursingii	France	1	0
Acinetobacter genomospecies 3	France	1	0
Acinetobacter genomospecies 9	France	3	3
	Germany	1	1
Acinetobacter genomospecies 10	France	1	0
Acinetobacter genomospecies 13	France	1	0
Acinetobacter genomospecies 15	France	1	0
Acinetobacter genomospecies 16	France	1	0
Acinetobacter genomospecies 17	France	1	0

[a] Place of origin and number of isolates for *A. baumannii* strains: France (23), Romania (3), Belgium (1), Turkey (1), Sweden (3), Tunisia (1), Tahiti (2), Benin (1), Libya (1), Vietnam (1), New Caledonia (2), South Africa (2), Egypt (1), Monaco (1), Réunion (1), United States (2), Switzerland (1), China (1), Russia (1), Spain (1).

[b] Number of isolates in which IS*Aba1*-like copies were detected.

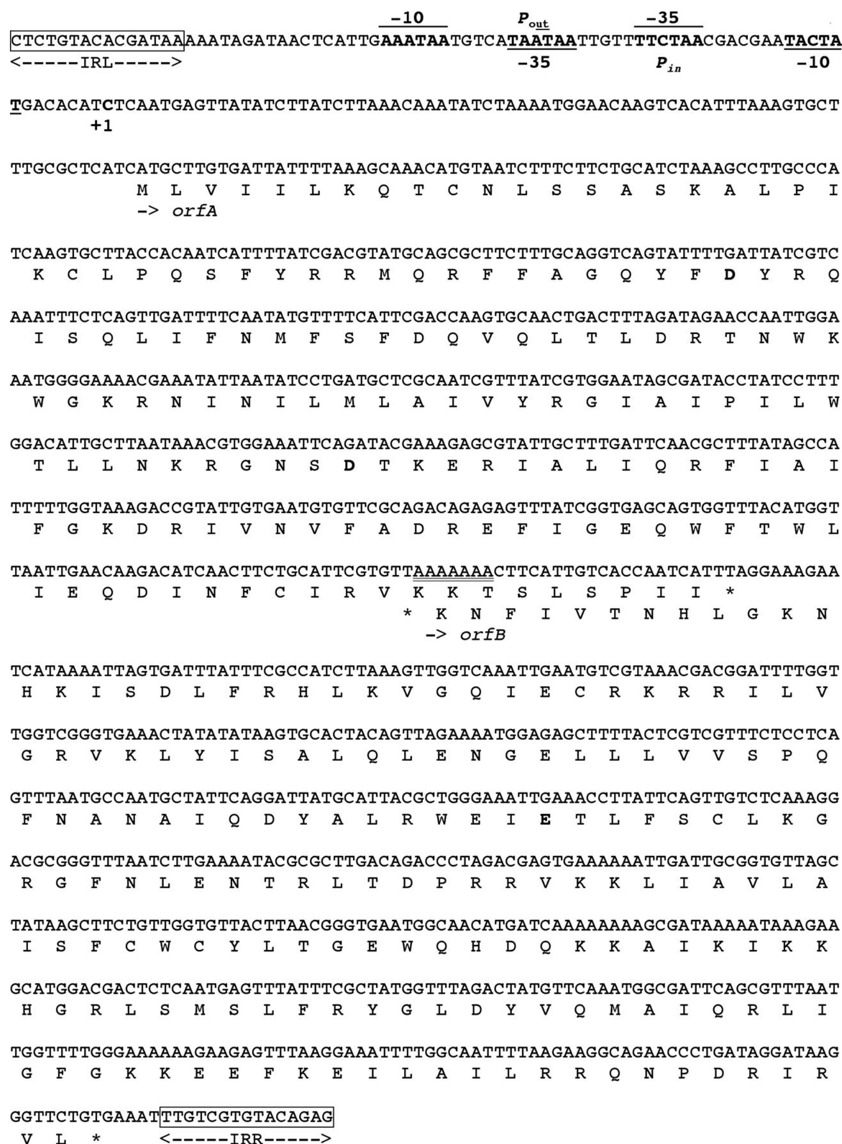

FIG. 1. Sequence of IS*Aba1*. The deduced amino acid sequence is designated by a single-letter code below the nucleotide sequence, and left and right inverted repeats (IRL and IRR, respectively) are boxed. The asterisks indicate stop codons. The −35 and −10 promoter boxes are indicated. P_{in} corresponds to the promoter of the *orfAB* transposase gene, and P_{out} to the promoter provided by IS*Aba1* for expression of adjacent genes. The +1 of transcription for *tnpA* is indicated in boldface type. The A_7 motif is underlined, and the DDE motif is boldfaced.

sition efficiency might result from a constitutive expression of the transposase. IS*Aba1* exhibits an A_7 motif that we showed to be responsible for a negative regulation of *tnpA* expression.

The genetics of acquisition of the bla_{OXA-23} gene had been previously investigated, and the composite transposon Tn*2006* was identified (7). The entire Tn*2006* sequence was cloned in pBK-CMV vector (kanamycin resistant), and transposition events were analyzed as described previously (14). The bla_{OXA-23} gene conferring resistance to amoxicillin provided a marker to follow transposition events. The transposition efficiency of Tn*2006* was found to be $(1.6 \pm 2.5) \times 10^{-8}$, being 10-fold lower than that observed with a single IS*Aba1* element, suggesting that IS*Aba1* transposition frequency might decrease with the length of the mobilized DNA fragment.

To demonstrate that the IS*Aba1* element possesses promoter sequences for *tnpA* gene expression, the site of transcription initiation for the *tnpA* gene was mapped from RNA of *A. baumannii* AYE (33), using the 5′ RACE (rapid amplification of cDNA ends) PCR technique (version 2.0; Invitrogen, Life Technologies, Cergy-Pontoise, France). The +1 transcription start was found to be 73 bp upstream of the start codon. The promoter P_{in} was subsequently defined to be made of the −10 (TACTAT) and −35 (TAATAA) boxes separated by 18 bp (Fig. 1). We showed here that the *tnpA* gene possesses promoter sequences, conferring on IS*Aba1* the property to be an autonomous mobile element.

In order to expand the knowledge related to IS*Aba1* distribution among the *Acinetobacter* genus, 14 different species of *Acinetobacter* were screened by PCR. IS*Aba1* was found in

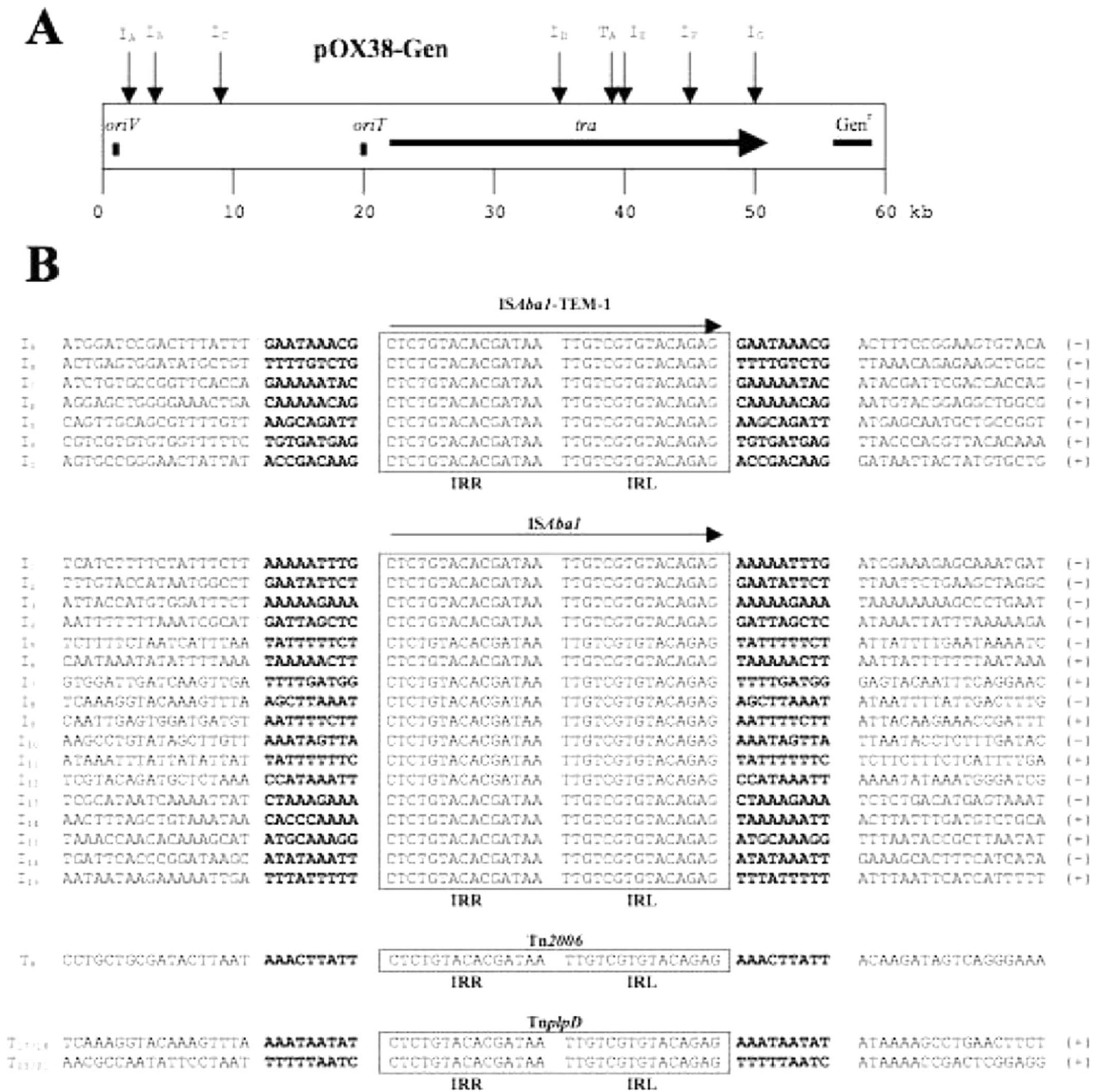

FIG. 2. Target sites of IS*Aba1* and Tn*2006* insertions. (A) Positions of the IS*Aba1*-TEM-1 and Tn*2006* insertions in plasmid pOX38-Gen. Insertions of the tagged IS (I_A to I_G) and of the composite transposons (T_A) are indicated by vertical arrows. The origin of replication (*oriV*), the origin of transfer (*oriT*), the *tra* genes required for plasmid transfer, and the gentamicin resistance gene (Gen^r) are indicated. (B) Nucleotide sequence alignments of the seven pOX38-Gen::IS*Aba1*-TEM-1 transconjugants and the 17 copies of IS*Aba1* in the *A. baumannii* AYE genome, pOX38-Gen::Tn*2006* transconjugants, and Tn*plpD* made by the 17 to 18 and 20 to 21 IS*Aba1* copy numbers and environment are shown. Nucleotide sequences of the end regions of IS*Aba*1-TEM-1, IS*Aba1*, and Tn*2006* are boxed. Boldfaced letters indicate target site sequences duplicated upon transposition. Gray boxes indicate conserved nucleotides in the environment of the IS*Aba1*-TEM-1 and Tn*2006* insertions. Orientation of the IS of IS*Aba1*-TEM-1, IS*Aba1*, and Tn*2006* is indicated by (+) and (−).

wild-type and in carbapenem-resistant *A. baumannii* isolates obtained from worldwide sources but also in five other *Acinetobacter* species (Table 3).

In silico analysis of the *A. baumannii* AYE genome (24, 33) identified 21 copies of IS*Aba1*, differing only by a single-base-pair substitution (guanine to adenine at position 65) located upstream of *orfA*, and therefore, modifying neither the coding sequence nor the promoter sequences of the transposase gene. IS*Aba1*-related gene disruption was found for different genes. Seven configurations in which IS*Aba1* was located close to and

upstream of genes were identified, likely providing promoter sequences enhancing their expression. Six copies of IS*Aba1* were in such configuration that they were forming composite transposons. Two copies (both bracketed by target site duplications) surrounded the *plpD* gene encoding a putative phospholipase D, and the same IS*Aba1*-*plpD*-IS*Aba1* structure was identified twice as a composite transposon named Tn*plpD* (Fig. 2). Two copies of Tn*plpD* had thus been very likely generated upon IS*Aba1*-mediated transposition, giving rise to a *plpD* multicopy. By analyzing in silico the recently available *A. baumannii* genome sequences, nine copies of IS*Aba1* were identified from strain AB0057 (1) and only a single copy was identified in strain ATCC 17978 (31), whereas IS*Aba1* was absent from strains AB307-0294, AB900 (1), SDF (33), and ACICU (11).

Here, we demonstrated that IS*Aba1* and the composite transposon Tn*2006* were capable of transposition in *E. coli* strains as well as the ability of IS*Aba1* to mobilize an antibiotic resistance gene. The IS*Aba1* element contains two ORFs, encoding a functional transposase, regulated by a mechanism named programmed translational frameshifting that has already been identified at least for IS*3* family members (4). This study reports on the very first functional properties of an IS element in *A. baumannii*.

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