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Transposons carrying the *aacC2e* aminoglycoside and *bla*$_{TEM}$ beta-lactam resistance genes in *Acinetobacter*

Liam A. Tobin[1], Amy K. Cain[2], Steven P. Djordjevic[1] and Mehrad Hamidian[1]*

[1] *Australian Institute for Microbiology and Infection, University of Technology Sydney, Ultimo, NSW Australia.*

[2] *ARC Centre of Excellence in Synthetic Biology, School of Natural Sciences, Macquarie University, Sydney, Australia*

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Authorship contribution statement

All authors declare that they have participated in the work and take public responsibility for the content. Conceptualization: MH, Data Curation LAT, MH; Formal Analysis: LAT, MH; Investigation: LAT, AKC, SPD, MH; Methodology: LAT, AKC, SPD, MH; Validation: AKC, SPD, MH; Visualization: LAT, AKC, SPD, MH; Writing – Original Draft: MH; Writing – Review & Editing: LAT, AKC, SPD, MH.

*__Corresponding author:__ Mehrad Hamidian

Mailing address: Australian Institute for Microbiology and Infection (AIMI),

University of Technology Sydney,

Ultimo, NSW, Australia.

Mobile: +61-421827161.

Email: mehrad.hamidian@gmail.com; mehrad.hamidian@uts.edu.au

Abstract

This study examines the genetic contexts and evolutionary steps responsible for the formation of the widely spread transposon Tn*6925* carrying *bla*$_{TEM}$ and *aacC2e,* which confers resistance to beta-lactam and aminoglycoside antibiotics in Gram-negative bacteria. The *bla*$_{TEM-1}$ and *aacC2e* genes were found in several transposons. They were first observed within an IS*26* bounded 3.7 kb transposon (Tn*6925)* on several *Acinetobacter baumannii* plasmids located within a 4.7 kb p*dif* module. Truncated and expanded variations of Tn*6925* were found across other *A. baumannii* plasmids, as well as in other Gram-negative bacteria (including *Vibrio cholerae*). Moreover, *bla*$_{TEM-1}$ and *aacC2e* were in much larger resistance-heavy transposons including the ISAba1 bounded 24.6 kb (here called Tn*6927*), found in an *A. baumannii* chromosome. A novel IS*Kpn12* bounded transposon was also observed to contain *bla*$_{TEM}$ and *aacC2e* which was found interrupting Tn*5393* along with an IS*26* pseudo-compound transposon (PCT) to form a 24.9 kb resistance island in an *Acinetobacter pittii* plasmid. Multiple mobile genetic elements are involved in the formation of transposon structures that circulate *bla*$_{TEM}$ and *aacC2e*. Amongst these, IS*26* and ISAba1 appear to have played a major role in the formation and spread of these elements in the *Acinetobacter* species.

Introduction

Antibiotic resistance continues to rise unabated, posing a significant public health threat. *Acinetobacter baumannii* is a particularly concerning pathogen because of its ability to develop resistance to last resort antibiotics.[1,2] One of the primary resistance mechanisms in Gram-negative bacteria, including *A. baumannii*, is the acquisition of antibiotic resistance genes via mobile genetic elements, including plasmids and transposons.[2-6] However, despite significant efforts to characterise the distribution and genetic context of clinically relevant antibiotic resistance genes, the involvement of numerous novel mobile genetic elements in the dissemination of resistance genes remains unexplored amongst *A. baumannii* clones.

Recently, we described Tn*6925*, a class 1 transposon embedded in a p*dif* module (DNA segment flanked by binding sites for XerC and XerD recombinases[7]) that was carried by variants of Rep_3 (R3-type) type plasmids[6] among ST1 and ST15 *A. baumannii* isolates recovered in Chile.[8] Tn*6925* is 3,748 bp in length, flanked by IS*26*, and contains the antibiotic resistance genes, *bla*$_{\text{TEM}}$ and *aacC2e*.[8] The *aacC2e* gene, which encodes an aminoglycoside acetyltransferase, is one of the most common genes associated with aminoglycoside resistance in Gram-negative bacteria.[9] It confers resistance to several aminoglycoside antibiotics, including gentamicin, tobramycin, kanamycin and neomycin.[9] In Enterobacterales, the *aacC2e* gene has been found on various incompatibility plasmid groups, including IncI1, IncB, IncF, IncHI1 and IncN.[9-12] However, the genetic structure of mobile elements that carry *bla*$_{\text{TEM}}$ and *aacC2e* has not been studied in the *Acinetobacter* genus.

The *bla*$_{\text{TEM}}$ gene encodes one of the most globally prevalent classes of extended-spectrum beta-lactamases (ESBL) found predominantly within Enterobacterales.[13,14] Of this class, *bla*$_{\text{TEM-1}}$, the first identified *bla*$_{\text{TEM}}$ variant, confers resistance to ampicillins – via beta-lactam hydrolysis– with some variants also conferring resistance to extended-spectrum beta-lactam antibiotics.[13,14] This resistance gene is typically plasmid-mediated and is a component of the wide-spread Tn*1-3* transposons.[13,14] Notably, *aacC2e* and *bla*$_{\text{TEM-1}}$ have been repeatedly observed to be associated with each other in a wide range of genetic contexts. In pSRC27-H, an IncHI1 plasmid found in

Salmonella enterica (GenBank accession number HQ840942), *aacC2e* and *bla*$_{TEM}$ form part of an IS*26*-flanked transposon with a mosaic structure containing ISCfr1, remnants of Tn*5393*, which is a well-studied transposon[15], and CR2, as well as two copies of the *bla*$_{TEM}$ end of Tn*2*.[12] In pRYC11, an IncFII$_k$ plasmid found in *Klebsiella pneumoniae* (GenBank accession number LK391770), *aacC2e* and *bla*$_{TEM}$ are part of a 10.5 kb variable region flanked by an IS*26*, IS*Kpn12* and a truncated ISEclI-like copy[11] indicating a complex evolutionary history.

Here, we explored the evolution of transposons that carry *aacC2e* and *bla*$_{TEM}$ outside Enterobacterales, in strains belonging to the *Acinetobacter* genus. Our findings provide new insights into the evolution and spread of *aacC2e* and *bla*$_{TEM}$ in the *Acinetobacter* genus, emphasising the role of insertion sequences in forming small transposons that facilitate their movement.

Methods

Genome and Plasmid sequence data

Genome sequences, including plasmid sequence data, were found in GenBank non-redundant database with all GenBank accession numbers either included in the text or Table 1. The original Tn*6925* sequences used to explore genome data are available under GenBank accession numbers CP076822.1 and CP076819.1.

Sequence analysis and bioinformatics

Mobile genetic elements were identified using several bioinformatics tools, including ISFinder (https://isfinder.biotoul.fr/blast.php) and Standalone BLAST, publicly available at https://www.ncbi.nlm.nih.gov/books/NBK52640/. Antibiotic resistance genes were identified using several tools and databases, including the Abricate software (available at https://github.com/tseemann/abricate), and the ResFinder (https://bio.tools/resfinder) and CARD (The Comprehensive Antibiotic Resistance Database;

https://card.mcmaster.ca/ontology/42600) databases. Protein coding regions were characterised using BLASTp (blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) and UniProt (https://www.uniprot.org/) searches. Putative plasmid replication initiation proteins were typed (and named) using the Acinetobacter Plasmid Typing (APT) scheme publicly available at GitHub (https://github.com/MehradHamidian/AcinetobacterPlasmidTyping). [5] The CheckM software was used (accessible at https://github.com/Ecogenomics/CheckM) to assess the quality of all publicly available genomes used in this study, as it offers reliable assessments of genome completeness and contamination.[16] The SnapGene® v6.0.5 software was used to manually annotate regions of interest and draw figures to scale using the Illustrator® v26.2.1 program.

Results and Discussion

*IS*26 *pseudo compound transposons containing* aacC2e *and* bla*TEM*

We previously reported that *aacC2e* and *bla*$_{TEM}$ are part of a pseudo-compound transposon (PCT) 3.7 kb transposon (Tn*6925*) formed by IS*26* in a set of ST1 and ST15 *A. baumannii* isolates recovered in Chile.[8] It was shown that Tn*6925* is flanked by eight (n=8) bp target site duplication (TSD) sequences typical of IS*26* transposition and consistent with Tn*6925* insertion (Figure 1). It has been shown that structures bounded by directly oriented members of the IS*26* family are pseudo-compound transposons (PCT) and can move via an intermolecular reaction catalysed by the IS-encoded transposase and an intramolecular homologous recombination step. [17] Further, Tn*6925* was found within a 4.6 kb p*dif* module in two different R3-type plasmids (encoding Rep_3), p1UC22850 (Figure 1; GenBank accession number CP076822.1) and p2UC24137 (GenBank accession number CP076819.1)[8], which also gives it the ability to move.

Here, we performed further analysis to track structures that include *aacC2e* and *bla*$_{TEM}$ to explore their evolution and identify mobile genetic elements that carry these antibiotic resistance genes. A third plasmid, (p1UC23022; GenBank accession number CP076813)

carried by a ST79 *A. baumannii* isolate from Chile includes a single copy of IS*26* precisely in the same position that Tn*6925* would be in p1UC22850 (Figure 1), an observation that explains how Tn*6925* has formed within this site. This insertion site is intact in pAb244_7 (Figure 1; GenBank accession number MG520098)[18], which is another related plasmid carried by the *A. baumannii* strain Ab244 that has its origins in Argentina. Tn*6925-v1*, a variant of Tn*6925* with an extra 128 bp end of IS*26* (Figure 1), was also found to be present in pMC75.2 (GenBank accession number MK531541.1), which is yet another R3-type *A. baumannii* plasmid. Further exploration of GenBank identified an additional variants of Tn*6925* in *A. baumannii* (named as Tn*6925-v2*) (Figure 2a). Tn*6925-v2* carries *accC2e* and is flanked by IS*26* but is only 2.4 kb in size and has lost bla_{TEM}, likely a result of an IS*26*-mediated adjacent deletion.[19] Notably, pAb825_36 (GenBank accession number MG100202)[18] has an additional beta-lactamase encoding gene ($bla_{\text{OXA-58}}$) compared to p1UC22850 (GenBank accession number CP076822), potentially indicating that it no longer requires the expression of bla_{TEM} to provide beta-lactam resistance. Tn*6925-v2* is also present in pAb825_36 (GenBank accession number MG100202), a 35.7 kb plasmid carried by *A. baumannii* Ab825 recovered from a wound sample in Argentina.[20] It appears that Tn*6925* and its variants can move the *aacC2e* and bla_{TEM} genes via the movement of the IS*26* PCT and recombination p*dif* module.

Another 4,121 bp region flanked by IS*26* carrying bla_{TEM} and *aacC2e* (Figure 2a), was found in a *Vibrio cholerae* plasmid (p2012EL-2176; GenBank accession number CP007636.1), however, was 373 bp longer compared to Tn*6925*. This region also includes a *res* gene (encoding a recombinase related to Tn*1-3*), which does not exist in Tn*6925* (Figure 2a) and therefore suggesting a different evolutionary path and origin (compared to Tn*6925*).

However, together this indicates that the spread of transposons carrying *aacC2e* and bla_{TEM} are not restricted to Enterobacterales and *Acinetobacter* and that IS*26* has played an important role in creating and moving IS*26*-formed structures that carry these genes in different genera.

*Tn*6927 – *a large composite transposon flanked by ISAba1*

aacC2e and *bla*$_{TEM}$ are also part of a large (24.6 kb) resistance region with a mosaic structure in several ST79 *A. baumannii* strains from Spain, Canada and Mexico (Table 1). In AbH12O-A2, in addition to *aacC2e* and *bla*$_{TEM}$, this large resistance region includes *sul2* (sulfonamide resistance), *aacA1* (aminoglycoside resistance), and *strAB* (streptomycin and spectinomycin resistance) genes (Figure 2b). Moreover, this structure contains several insertion sequences, including IS*1006*, ISAba43 and an IS*91*-variant, and remnants of other IS, including ISvsa3 and ISKpn11. The entire structure is bounded by two ISAba1 copies and flanked by nine (n=9) bp TSD. This indicates that the transposition of this novel structure, here named Tn*6927* (Figure 2b), occurred as a transposon unit into the chromosome of AbH12O-A2 at base 29,683 of GenBank accession number CP009534.1, between LX00_17455 encoding a hypothetical protein and LX00_17605 encoding NAD(P)H oxidoreductase. Tn*6927* and its deletion variant were also found in *A. baumannii* strains AB030 and AF-401 recovered in Canada and Mexico, respectively (Table 1), indicating its wide geographical distribution. The geographical distribution and isolation date (2006-2010) of ST79 strains (Table 1), suggests that the acquisition of Tn*6927* is likely to have occurred in an ancestral strain prior to 2006.

*Tn*5393*::Tn*Kpn12*::IS*26*PCT – a complex structure found in an Acinetobacter pittii *plasmid*

Further analysis performed here led to the characterisation of yet another mosaic resistance element carrying *aacC2e* and *bla*$_{TEM}$ in an *Acinetobacter pittii* plasmid pAB17H194-1 (GenBank accession number CP040912.1; Table 1). Detailed analysis revealed that this 25 kb structure is a Tn*5393* transposon that has been interrupted by two other transposon structures (Figure 2c). The *bla*$_{TEM}$ and *aacC2e* genes are part of a structure bounded by IS*Kpn12* interrupting *strA*. This insertion, here referred to as Tn*Kpn12*, appears to have generated two (n=2) bp TSD, compared to an intact *strA* gene, suggesting transposition of Tn*Kpn12* to this position. The second insertion, in the backbone of Tn*5393*, was found to be an IS*26* PCT

(pseudo-compound transposon[17]) containing *aphA1* (kanamycin/neomycin resistance) and *msr-mph*(E) (macrolide resistance). Additionally, this structure was found interrupting the *tnpA* gene of Tn*5393* (Figure 2c). Consistent with the transposition of the IS*26* PCT to this position, an eight (n=8) bp (CTCGCGAT) TSD was also found flanking this region, indicating that the addition of the IS*26* PCT is likely to have been a more recent event, as Tn*5393* can no longer move given that its *tnpA* gene is interrupted.

Tn*5393* has been in circulation in diverse bacterial genera including *Pseudomonas, Xanthomonas, Erwinia, Snodgrassella* and *Aeromonas*.[21] Its global success has in part been linked with widespread use of aminoglycosides particularly in horticulture.[22] It is important to understand that the multiple drug resistant *Acinetobacter* described here, and indeed other clinically important bacterial species[15,22], have captured the key antibiotic resistance gene remnants of this transposon into complex resistance regions that are often located on plasmids. This observation underscores the importance of understanding how MDR lineages of *Acinetobacter* evolve and where the genetic components that comprise these regions are likely to have originated, particularly in light of recent reports of zoonoses involving *Acinetobacter*.[23]

Integrative elements

We recently reported a complex chromosomal resistance region in an Australian *A. baumannii* (strain RCH52; Table 1) that contained several antibiotic resistance genes, including *bla*$_{\text{TEM}}$ and *aacC2e*.[24] This 129 kb segment was bounded by inversely oriented copies of ISAba1 and included two groups of resistance genes separated by a large segment of the backbone of type 1 IncC plasmids.[24] The ISAba1-bounded segment was in a novel integrative element (IE) integrated into the chromosome of strain RCH52.

Here, we found two additional structures in plasmids pCCBH31270 (Figure 2b) and pCCBH31258 (GenBank accession numbers CP101886.1 and CP101888.1, respectively; Table 1) that contain the *aac2e-bla*$_{\text{TEM}}$ region shared with RCH52 (shared segment shown in Figure

2b) but in an integrative element unrelated to the integrative element found in strain RCH52. *A. baumannii* strains CCBH31270 and CCBH31258 that carry the two plasmids (pCCBH31270 and pCCBH31258) were recovered in Brazil in 2021. The presence of a shared *aac2e-bla*TEM region identified in pCCBH31258, Tn*6927* (Figure 2b), RCH2 chromosome[24] (not shown here), and similar regions found in Enterobacterales' plasmids suggests a common ancestor. However, this needs to be confirmed as more genomes become available.

Conclusions

The findings of this study indicate that a diverse range of mobile elements are involved in the spread of *aacC2e* and bla_{TEM} and that they have a complex evolutionary history. The transposons found here facilitate the spread of *aacC2e* and bla_{TEM} and contribute to the emergence and spread of aminoglycoside resistance in strains of the *Acinetobacter* genus and other genera such as *Vibrio*. As more genomes are sequenced, more intermediate structures will be identified shedding more light on their origin and complex evolutionary history.

Disclosure Statement

All authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the article; or in the decision to publish the results.

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Supplementary material

None.

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Table 1. Properties of genomes carrying transposons containing the *aacC2e* and *bla*$_{TEM}$ [a]

Genus/species	strain	Country	Isolation date	ST[IP]	Chromosome/ plasmid	Genetic context	GenBank Accession no.
A. baumannii	AbH12O-A2	Spain	2006-08	79	chromosome	Tn*6927*	CP009534.1
A. baumannii	AB030	Canada	2010	79	chromosome	Tn*6927*	CP009257.1
A. baumannii	AF-401	Mexico	2009	79	chromosome	Tn*6927-v1* [b]	CP018254.1
A. baumannii	RCH52	Australia	<2014	729[c]	chromosome	IE [d]	CP085788.1
A. baumannii	UC22850	Chile	2011	1	p1UC22850	Tn*6925*	CP076822.1
A. baumannii	UC24137	Chile	2011	15	p2UC24137	Tn*6925*	CP076819.1
A. baumannii	Acb-45063[e]	Brazil	2010	15	pAb45063_a	Tn*6925*	MK323042.1
A. baumannii	MC75	Bolivia	2016	15	pMC75.2	Tn*6925-v1*	MK531541.1
A. baumannii	Ab825	Argentina	1999	15	pAb825_36	Tn*6925-v2*	MG100202.1
A. baumannii	CCBH31270	Brazil	2021	- [c]	pCCBH31270	IE	CP101886.1
A. baumannii	CCBH31258	Brazil	2021	- [c]	pCCBH31258	IE	CP101888.1
A. pittii	AB17H194	China	2017	-	pAB17H194-1	Tn*5393*::Tn*Kpn12*::IS*26* PCT	CP040912.1
Vibrio cholerae	2012EL-2176	Haiti	2012	-	p2012EL-2176	Tn*6925-v3*	CP007636.1

[a] Those present in IncC plasmids are not included in this Table.

[b] Tn*6927*^{var1}: missing 98 bp (52 bp preceding the start codon of *aacA1*)

[c] SLV (single locus variant) of ST3.

[c] Could not be determined, missing one or more alleles likely due to deletion and/or assembly issues.

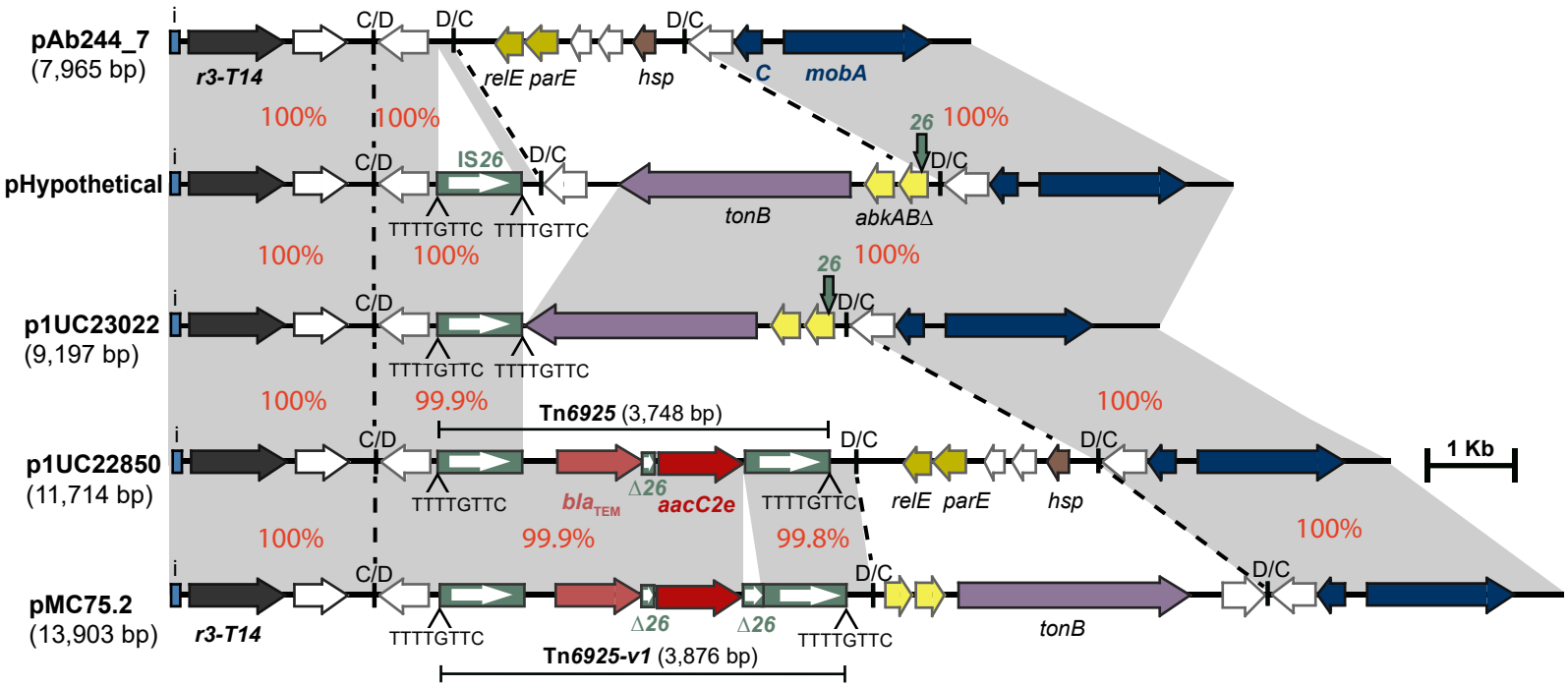
[d] Integrative Element.

[d] Described in [25].

Figure legends

Figure 1. Genetic structure of the composite transposons carrying the *bla*$_{TEM}$ and *aacC2e*. Filled arrows indicate the orientation and extent of genes. Resistance genes are colored red and the filled boxes colored green indicate insertion sequences. Black arrows are putative replication initiation genes and toxin/anti-toxin genes are yellow. Vertical black lines indicate p*dif* sites. The scale bar is shown. The red numbers within grey shaded areas indicate the percentage of DNA identity between the corresponding segments.

Figure 2. Genetic environments of mobile genetic elements that carry the *bla*$_{TEM}$ and *aacC2e* resistance genes in *A. baumannii* and *V. cholerae*. Arrows indicate genes with those colored red being antibiotic resistance genes. Green filled boxes indicate insertion sequences. **a)** indicates variants of composite transposons derived from Tn*6925* including Tn*6925-3* found in *V. cholerae*. **b)** illustrates the complex structure of Tn*6927*, which is flanked by two ISAba1 copies (light green boxes) with 9 bp target site duplications (AGGCAAAAT) shown on either side. Regions marked with red zigzagged lines indicate segments with significant homology to those found in Enterobacterales plasmids (e.g. IncC) and that recently described in *A. baumannii* RCH52. **c)** indicates the structure of the Tn*5393*::Tn*Kpn12*::IS*26* with a Tn*5393* transposon backbone interrupted by a Tn*Kpn12* copy and an IS*26* pseudo-compound transposon (PCT) carrying an *aphA1* and *mph-msr*(E) copies. The position of the GTTAA target site duplication flanking the Tn*5393*::Tn*Kpn12*::IS*26* is also shown. The red numbers within grey shaded areas indicate the percentage of DNA identity between the corresponding segments.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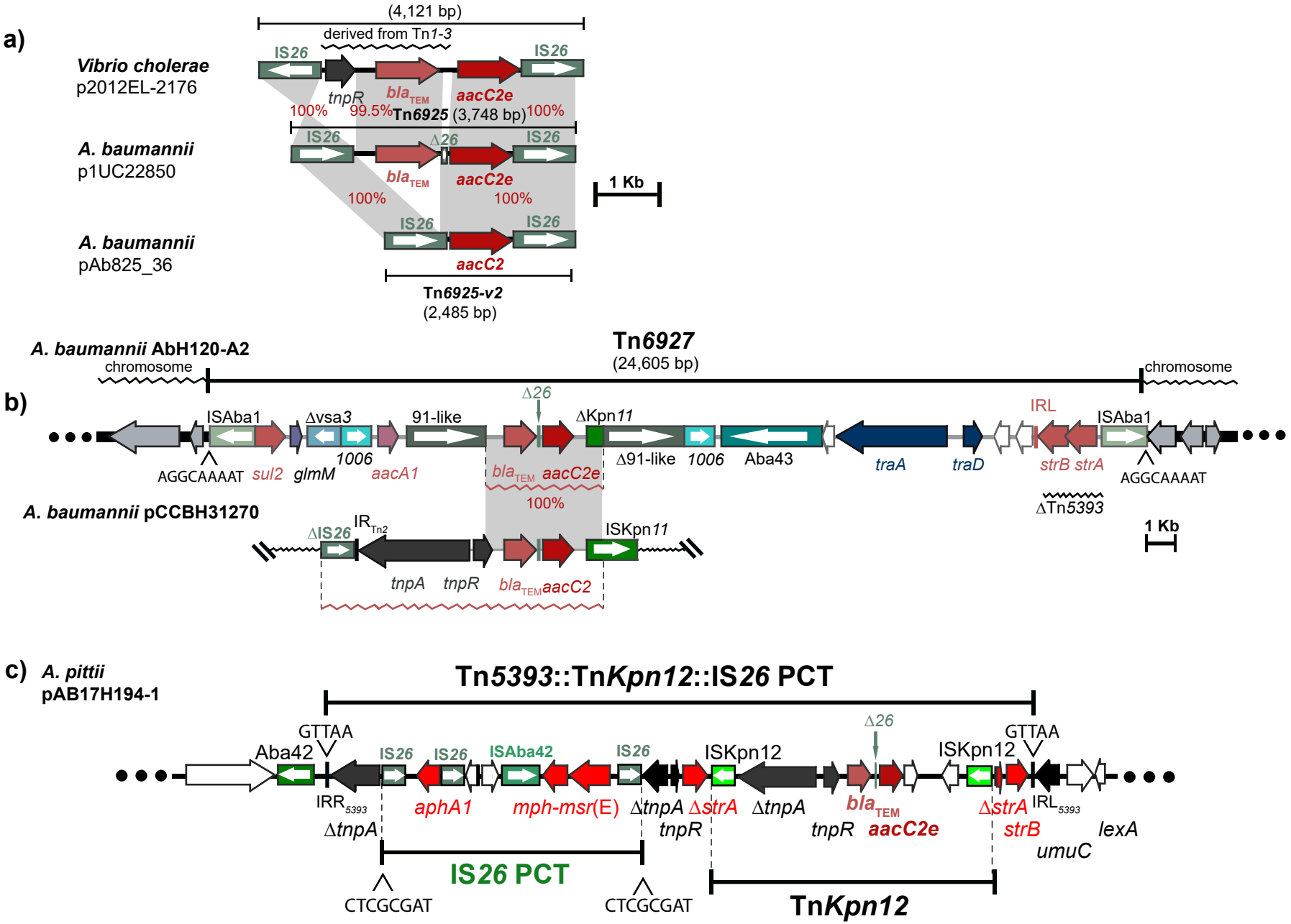
(7,965 bp)
r3-T14
relE parE
hsp
C
mobA
pHypothetical
IS26
tonB
abkAB∆
p1UC23022
(9,197 bp)
p1UC22850
(11,714 bp)
Tn6925 (3,748 bp)
bla TEM
∆26
aacC2e
relE parE
hsp
1 Kb
pMC75.2
(13,903 bp)
r3-T14
tonB
Tn6925-v1 (3,876 bp)
TTTTGTTC
C/D
D/C
100%
99.9%
99.8%


a)
Vibrio cholerae
p2012EL-2176
A. baumannii
p1UC22850
A. baumannii
pAb825_36
(4,121 bp)
derived from Tn1-3
IS26
tnpR
bla TEM
aacC2e
100%
99.5%
Tn6925 (3,748 bp)
Δ26
1 Kb
aacC2
Tn6925-v2
(2,485 bp)
b)
A. baumannii AbH120-A2
chromosome
Tn6927
(24,605 bp)
ISAba1
Δvsa3
91-like
ΔKpn11
IRL
AGGCAAAAT
sul2
glmM
1006
aacA1
Δ91-like
Aba43
traA
traD
strB strA
ΔTn5393
A. baumannii pCCBH31270
ΔIS26
IR Tn2
ISKpn11
tnpA
tnpR
bla TEM aacC2
c)
A. pittii
pAB17H194-1
Tn5393::TnKpn12::IS26 PCT
GTTAA
Aba42
ISAba42
ISKpn12
IRR 5393
aphA1
mph-msr(E)
ΔtnpA
ΔstrA
IRL 5393
strB
lexA
umuC
IS26 PCT
CTCGCGAT
TnKpn12