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Detection of Gene Cassettes in Tn*402*-Like Class 1 Integrons[▽]

Amplification of the gene cassettes in class 1 integrons by PCR using primers in the 5′ conserved segment (5′-CS) and the 3′-CS (7, 8) has been used in hundreds of studies to identify integron-associated resistance genes (Fig. 1A). Equivalent PCR primers that detect cassettes in class 2 integrons, i.e., Tn*7* family transposons (18), are also widely used. The approach used to detect integrons in antibiotic-resistant bacteria is to screen for the *intI* genes, using primers internal to these genes, and then amplify the cassettes in *intI*-positive strains by use of primers in the flanking conserved regions. Because different cassettes can have the same size and the arrays can include more than one gene (16), amplicon size alone cannot identify the cassettes, which are characterized by sequencing, PCR mapping (7), or restriction fragment polymorphisms (9, 10).

However, for some *intI1*-positive strains, a cassette PCR amplicon is not observed, and to date these strains have been largely ignored, even though such isolates can represent a significant proportion of the isolates studied (3). For class 1 integrons, if the *sul1* gene found in the 3′-CS in both of the main structural types (12, 13) is not present, the absence of a PCR product may indicate that the priming site in the 3′-CS is missing. This can occur because the integron is recombinant with the 5′-CS of class 1 and the *tns* module of class 2 (14). Additionally, the 3′-CS is not found in Tn*402* (15), the likely

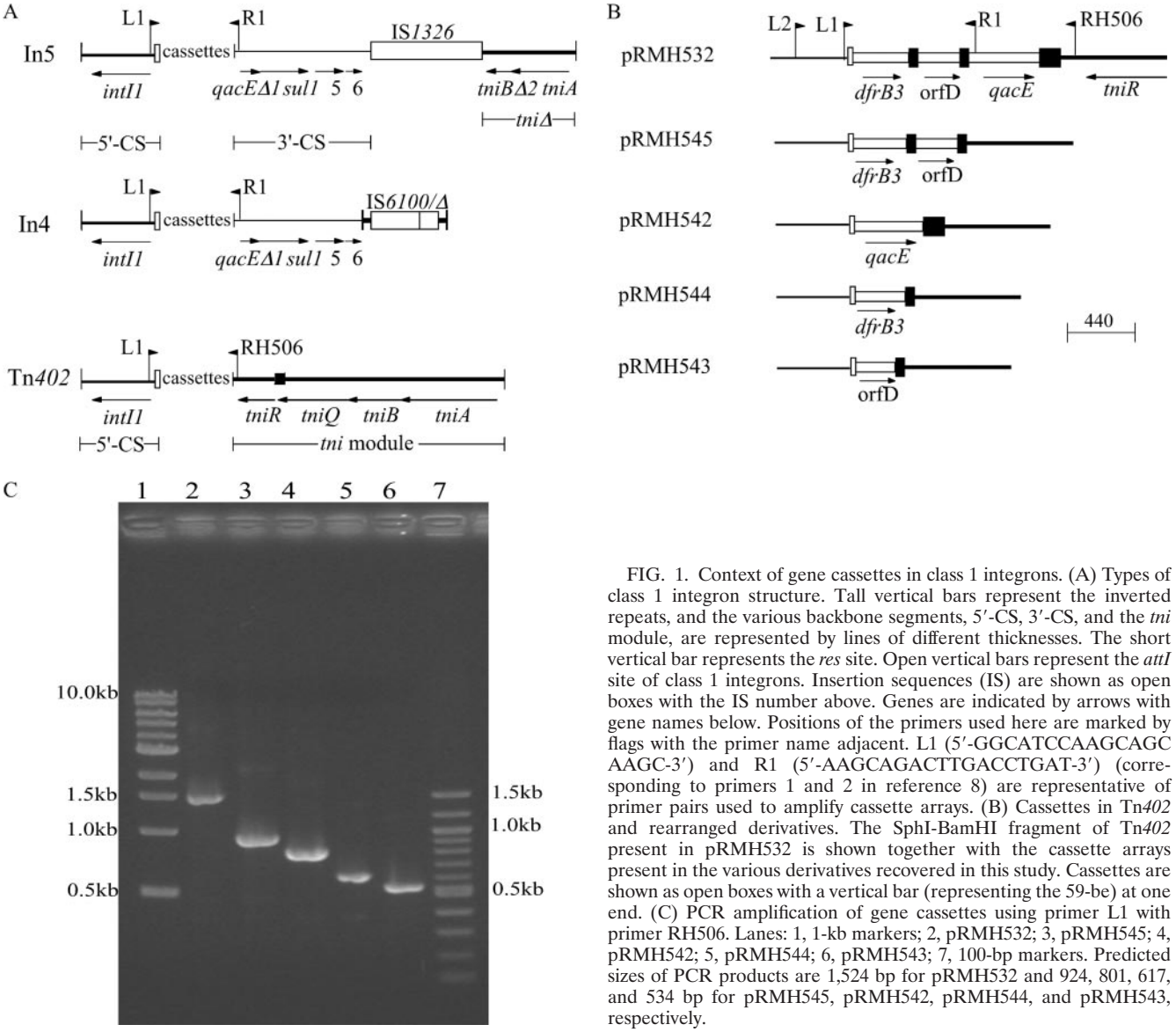

FIG. 1. Context of gene cassettes in class 1 integrons. (A) Types of class 1 integron structure. Tall vertical bars represent the inverted repeats, and the various backbone segments, 5′-CS, 3′-CS, and the *tni* module, are represented by lines of different thicknesses. The short vertical bar represents the *res* site. Open vertical bars represent the *attI* site of class 1 integrons. Insertion sequences (IS) are shown as open boxes with the IS number above. Genes are indicated by arrows with gene names below. Positions of the primers used here are marked by flags with the primer name adjacent. L1 (5′-GGCATCCAAGCAGC AAGC-3′) and R1 (5′-AAGCAGACTTGACCTGAT-3′) (corresponding to primers 1 and 2 in reference 8) are representative of primer pairs used to amplify cassette arrays. (B) Cassettes in Tn*402* and rearranged derivatives. The SphI-BamHI fragment of Tn*402* present in pRMH532 is shown together with the cassette arrays present in the various derivatives recovered in this study. Cassettes are shown as open boxes with a vertical bar (representing the 59-be) at one end. (C) PCR amplification of gene cassettes using primer L1 with primer RH506. Lanes: 1, 1-kb markers; 2, pRMH532; 3, pRMH545; 4, pRMH542; 5, pRMH544; 6, pRMH543; 7, 100-bp markers. Predicted sizes of PCR products are 1,524 bp for pRMH532 and 924, 801, 617, and 534 bp for pRMH545, pRMH542, pRMH544, and pRMH543, respectively.

TABLE 1. Cassette arrays flanked by the 5′-CS and *tni* modules

Organism	Gene cassettes	GenBank accession no.	Reference
Klebsiella aerogenes	*dfrB3*-orfD-*qacE*	X72585	15
Klebsiella pneumoniae	Unknown	U95134	
Pseudomonas aeruginosa	bla_{VIM2}-*oxa2-aacA4-aadB-qacG*	AY507153	
Pseudomonas aeruginosa	*aacA7-*bla_{VIM2}-*dfrB5-aacC-A5*	AY943084, DQ522233	11
Uncultured	*oxa2-aacA4-aadA1*	AJ744860	17
Pseudomonas aeruginosa	bla_{VIM2}-*aacA4*	AM180753	6
Pseudomonas aeruginosa	*aacA7-*bla_{VIM-2}-*dfrB5-aac6-II* (ISPa21-like)	AM296017	

progenitor of class 1 integrons (12, 13). Instead, Tn*402* includes a transposition gene module (*tni* module) containing *tniA*, *tniB*, *tniQ*, and *tniR* (also called *tniC*) (4, 5) (Fig. 1A). Several class 1 integrons with the *tni* module of Tn*402* but a variety of gene cassettes have been reported (Table 1).

To validate a method to detect cassettes in Tn*402*-type integrons, a fragment containing the cassette array of Tn*402* from R751 was cloned into pACYC184, generating pRMH532 (Fig. 1B). IntI1-generated derivatives of pRMH352 that had lost one or more of the gene cassettes (Fig. 1B) were constructed as described previously (1, 2). Digestion with SphI and BamHI and sequencing were used to identify the cassettes and establish the mobility of the 587-bp *qacE* cassette, partial copy (bp 1 to 390) of which is in the standard 3′-CS. The cassette arrays were amplified using the 5′-CS primers L1 (5′-GGCA TCCAAGCAGCAAGC-3′) (8) and L2 (5′-GACGATGCGT GGAGACC-3′) with primer RH506 (5′-TTCAGCCGCATA AATGGAG-3′) in the *tniR* gene (Fig. 1B). Plasmid DNA was prepared using a Wizard Plus SV Miniprep DNA purification kit (Promega). PCR amplification was carried out in PCR buffer (New England Biolabs) containing 160 μM of each deoxynucleotide triphosphate, 50 pmol of each primer, approximately 10 to 50 ng of template, and 1 unit of *Taq* DNA polymerase (Roche). Reaction conditions were 95°C for 3 min followed by 35 cycles of 95°C for 30 s, 64°C for 90 s, and 72°C for 90 s and by a final incubation at 72°C for 5 min. The sizes of the amplicons obtained with both L1 (Fig. 1C) and L2 and of RsaI restriction fragments were as predicted from the Tn*402* sequence (GenBank accession no. X72585; Tn*5090* is Tn*402*). This method should prove useful in analyzing strains that include *intI1* but not *sul1* and do not form an amplicon with the L1-R1 (or equivalent) primer pair.

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Virginia Post
School of Molecular and Microbial Biosciences
The University of Sydney, 2006
New South Wales, Australia

Gavin D. Recchia
Davies Collison Cave
255 Elizabeth Street
Sydney, 2000, New South Wales, Australia

Ruth M. Hall*
School of Molecular and Microbial Biosciences
The University of Sydney, 2006
New South Wales, Australia

*Phone: 61-29351-3465
Fax: 61-29351-4571
E-mail: ruth.hall@mmb.usyd.edu.au

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