Detection of Gene Cassettes in Tn402-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., Tn7 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 Tn402 (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 intI module, are represented by lines of different thicknesses. The short vertical bar represents the resistase 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′-GGCATCCAAGCAGCAAGC-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 Tn402 and rearranged derivatives. The SphI-BamHI fragment of Tn402 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 5′-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.
progenitor of class 1 integrons (12, 13). Instead, Tn402 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 Tn402 but a variety of gene cassettes have been reported (Table 1).

To validate a method to detect cassettes in Tn402-type integrons, a fragment containing the cassette array of Tn402 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 5′-3′ CS cassette array, 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 TCCAAGCAGCAACG-3′) (8) and L2 (5′-GACGATGCGT CAGCAGCAAGC-3′) with primer RHS06 (5′-TTCAGCCGCATA AATGGGACG-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 deoxyribonucleotide 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 Rsal restriction fragments were as predicted from the Tn402 sequence (GenBank accession no. X72585; Tn5090 is Tn402).

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.

REFERENCES


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

Gavin D. Recchia
Davies Collison Cave
253 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

Published ahead of print on 25 June 2007.