Gene Cassette Encoding a 3-*N*-Aminoglycoside Acetyltransferase in a Chromosomal Integrin

The large number and variety of antibiotic resistance genes found in gene cassettes associated with class 1 integrons in multiply antibiotic-resistant organisms have always indicated the existence of a large pool of gene cassettes from which they are drawn (10). The discovery of integrons, sometimes harboring very large arrays of gene cassettes, in the chromosomes of many different bacteria has confirmed the existence of this pool, but to date very few cassettes containing resistance genes have been found among these cassettes. Known examples are the *catB9* (12) and *blaP7* (*blaP9*) (7, 8) cassettes found in the cassette arrays of different *Vibrio cholerae* strains.

Though 3-*N*-aminoglycoside acetyltransferases [AAC(3) enzymes] have been classified into several groups based on differences in the spectrum of aminoglycosides they modify, they fall into only two groups based on the relationships between the proteins (6). The five characterized *aacC* genes found in gene cassettes (see Table 1) encode proteins of 154 to 158 amino acids that are related to one another and form family A (6). They belong to the *aac*(3)-I phenotypic group and confer resistance to gentamicin, sisomicin, and fortimicin but not to tobramycin, amikacin, or kanamycin (1, 3, 6, 11, 13, 15).

Our recent searches have revealed further potential *aacC*-A-type genes (Table 1). One is in a cassette found in a class 1 integron, but three of them are in draft sequences of bacterial genomes. The *aacC*-A7 gene in the complete *Saccharophagus*

degradans (formerly *Microbulbifer degradans*) genome also lies within an identifiable gene cassette. The putative *AacC*-A7 [AAC(3)-Ig] acetyltransferase encoded by this cassette is 51 to 60% identical to other cassette-encoded *AacC*-A enzymes and most closely related to *AacC*-A5 [AAC(3)-Ie]. Alignment of the established and putative *AacC*-A sequences (Fig. 1) reveals 45 completely conserved amino acids, or 62 if only *AacC*-A1 to *AacC*-A7 are considered. It therefore seems reasonable to assume that *aacC*-A7 also confers resistance to aminoglycoside antibiotics.

This represents only the third identification of a potential antibiotic resistance gene associated with a gene cassette that is part of the genome of a microorganism that harbors an integron. Though each *aacC*-A cassette has an identifiable 59-be (sometimes called an *attC* site) made up of two simple sites and a central region as is characteristic for 59-be (14), in most cases they are not close relatives of one another and some are not closely related to ones found in any other known cassette (6, 11). The 59-be of the *Saccharophagus aacC*-A7 cassette is not closely related to a 59-be in any of the many cassettes in that organism's genome. Its closest relative is in a cassette from *Nitrosococcus oceani*. Thus, if, as is generally assumed, cassettes that arose in a particular organism have very similar 59-be, it is unlikely that the *aacC*-A7 cassette was originally formed in *S. degradans*.

FIG. 1. Alignment of proteins in the AacC-A [AAC(3)-I] family. Amino acids completely conserved in all sequences are shown as white on black and are indicated by uppercase letters below the sequence. Amino acids conserved in 9 of the 10 sequences are indicated by lowercase letters below. The sequences of AacC-A proteins were obtained from the GenBank accession numbers listed in Table 1.

TABLE 1. *aacC-A* genes and gene cassettes

Gene name ^a	Previous name(s)	Length (bp)		Integron type	GenBank accession no.	Reference(s)
		Cassette	59-be			
<i>aacC-A1</i>	<i>aacC1</i> (<i>aac(3)-Ia</i>)	577	109	Class 1	X15852	15
<i>aacC-A2</i>	<i>aac(3)-Ib</i>	>498	>33	Class 1	L06157	13
<i>aacC-A3</i>	<i>aac(3)-Ic</i>	590	112	Class 1	AJ511268	11
<i>aacC-A4</i>	<i>aacC4</i>	576	108	Class 1	AF318077	4, 9
<i>aacC-A5</i>	<i>aac(3)-Id^b</i>	564	78	Class 1	AY463797	1, 6
<i>aacC-A6</i>		577	112	Class 1	AY884051	
<i>aacC-A7</i>		558	75	Genomic	CP000282 ^d	
<i>aacC-A8</i>		NA ^c			None ^e	
<i>aacC-A9</i>		NA ^c			None ^f	
<i>aacC-A10</i>		NA ^c			CP000155	

^a Numbers have been assigned in the order of release of the GenBank record (5). Only A1 to A5 have been demonstrated to confer aminoglycoside resistance.

^b The designation *-Id* for this gene (GenBank accession numbers AB114632, AY458224, and AY563051) is incorrect (1–3).

^c NA, not applicable (because this gene is not part of an identifiable cassette).

^d Genome of *Saccharophagus* (formerly *Microbulbifer*) *degradans* 2-40. Cassette is at 2,333,600 to 2,334,159 bp.

^e Two copies (98% identical) are found in the draft genome of *Paracoccus denitrificans* PD1222, U.S. Department of Energy Joint Genome Institute (the sequence can be obtained at http://genome.jgi-psf.org/draft_microbes/parde/parde.home.html or via GenBank accession no. AAIT000000000).

^f Found in the draft genome of *Sphingopyxis alaskensis* RB2256, U.S. Department of Energy Joint Genome Institute (the sequence can be obtained at http://genome.jgi-psf.org/draft_microbes/sphal/sphal.home.html or via GenBank accession no. AAI000000000).

REFERENCES

- Ahmed, A. M., T. Nakagawa, E. Arakawa, T. Ramamurthy, S. Shinoda, and T. Shimamoto. 2004. New aminoglycoside acetyltransferase gene, *aac(3)-Id*, in a class 1 integron from a multiresistant strain of *Vibrio fluvialis* isolated from an infant aged 6 months. *J. Antimicrob. Chemother.* **53**:947–951.
- Cabrera, R., J. Ruiz, F. Marco, I. Oliveira, M. Arroyo, A. Aladuena, M. A. Usera, M. T. Jimenez De Anta, J. Gascon, and J. Vila. 2004. Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing traveler's diarrhea. *Antimicrob. Agents Chemother.* **48**:3934–3939.
- Doublet, B., F. X. Weill, L. Fabre, E. Chaslus-Dancla, and A. Cloeckaert. 2004. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3'-N-aminoglycoside acetyltransferase gene cassette, *aac(3)-Id*, in *Salmonella enterica* serovar Newport. *Antimicrob. Agents Chemother.* **48**:3806–3812.
- Gibb, A. P., C. Tribuddharat, R. A. Moore, T. J. Louie, W. Krulicki, D. M. Livermore, M. F. I. Palepou, and N. Woodford. 2002. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new *bla*_{IMP-7} allele, *bla*_{IMP-7}. *Antimicrob. Agents Chemother.* **46**:255–258.
- Hall, R., and S. Partridge. 2003. Unambiguous numbering of antibiotic resistance genes. *Antimicrob. Agents Chemother.* **47**:3998–3999.
- Levings, R. S., S. R. Partridge, D. Lightfoot, R. M. Hall, and S. P. Djordjevic. 2005. New integron-associated gene cassette encoding a 3-N-aminoglycoside acetyltransferase. *Antimicrob. Agents Chemother.* **49**:1238–1241.
- Melano, R., A. Petroni, A. Garutti, H. A. Saka, L. Mange, F. Pasterán, M. Rapoport, A. Rossi, and M. Galas. 2002. New carbenicillin-hydrolyzing β -lactamase (CARB-7) from *Vibrio cholerae* non-O1, non-O139 strains encoded by the VCR region of the *V. cholerae* genome. *Antimicrob. Agents Chemother.* **46**:2162–2168.
- Petroni, A., R. G. Melano, H. A. Saka, A. Garutti, L. Mange, F. Pasterán, M. Rapoport, M. Miranda, D. Faccione, A. Rossi, P. S. Hoffman, and M. F. Galas. 2004. CARB-9, a carbenicillinase encoded in the VCR region of *Vibrio cholerae* non-O1, non-O139 belongs to a family of cassette-encoded β -lactamases. *Antimicrob. Agents Chemother.* **48**:4042–4046.
- Poirol, L., G. F. Weldhagen, C. De Champs, and P. Nordmann. 2002. A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended-spectrum β -lactamase GES-2 in South Africa. *J. Antimicrob. Chemother.* **49**:561–565.
- Recchia, G. D., and R. M. Hall. 1997. Origins of the mobile gene cassettes found in integrons. *Trends Microbiol.* **5**:389–394.
- Riccio, M. L., J. D. Docquier, E. Dell'Amico, F. Luzzaro, G. Amicosante, and G. M. Rossolini. 2003. Novel 3-N-aminoglycoside acetyltransferase gene, *aac(3)-Ic*, from a *Pseudomonas aeruginosa* integron. *Antimicrob. Agents Chemother.* **47**:1746–1748.
- Rowe-Magnus, D. A., A. M. Guerout, and D. Mazel. 2002. Bacterial resistance evolution by recruitment of super-integron gene cassettes. *Mol. Microbiol.* **43**:1657–1669.
- Schwocho, L. R., C. P. Schaffner, G. H. Miller, R. S. Hare, and K. J. Shaw. 1995. Cloning and characterization of a 3-N-aminoglycoside acetyltransferase gene, *aac(3)-Ib*, from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **39**:1790–1796.
- Stokes, H. W., D. B. O'Gorman, G. D. Recchia, M. Parsekhian, and R. M. Hall. 1997. Structure and function of 59-base element recombination sites associated with mobile gene cassettes. *Mol. Microbiol.* **26**:731–745.
- Wohlleben, W., W. Arnold, L. Bissonnette, A. Pelletier, A. Tanguay, P. H. Roy, G. C. Gamboa, G. F. Barry, E. Aubert, J. Davies, and S. A. Kagan. 1989. On the evolution of Tn21-like multiresistance transposons: sequence analysis of the gene (*aacC1*) for gentamicin acetyltransferase-3-I (AAC(3)-I), another member of the Tn21-based expression cassette. *Mol. Gen. Genet.* **217**:202–208.

Liam D. H. Elbourne
School of Molecular and Microbial Biosciences
The University of Sydney
Sydney, NSW 2006, Australia

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

*Phone: 61-2-9351-4365
Fax: 61-2-9351-4571
E-mail: Ruth.Hall@mmb.usyd.edu.au