If analysis of the gene cassettes located in class 1 or class 2 integrons found in surveys of antibiotic resistance genes is to be useful in tracking the prevalence and movement of resistance genes, the cassettes must be accurately identified. This can be simply achieved by citing the GenBank accession numbers for the relevant reference cassettes listed in available compilations (e.g., references 6 and 9). However, confusion is arising around the single cassette known to include a streptothricin acetyltransferase gene (GenBank accession no. X15995), which was first identified in transposons Tn1825 and Tn1826 isolated from bacteria resistant to streptothricin (4, 12). These transposons are close relatives of Tn7 (14), and the sat cassette was subsequently identified in Tn7 (3, 11, 13), where it confers only modest levels of resistance to the antibiotic (11, 13). Thus, Tn7, Tn1825, and Tn1826 are all class 2 integrons that appear to differ only in the composition of the cassette array (see reference 9) (Fig. 1A).

The confusion has arisen because proteins of different sizes were produced by the cloned streptothricin resistance determinants of Tn1825 and Tn1826, and the genes were named sat1 and sat2 (15). Tn1826, in which the sat cassette is first in the cassette array, produces the shorter Sat-2 protein, which is sufficient to confer streptothricin resistance. In Tn1825 the sat cassette is preceded by another previously unnamed cassette (estX in Fig. 1), and the protein produced by this cassette is fused to Sat-2, forming the longer Sat-1 (15). Further sequences of cassette arrays equivalent to that of Tn1825 are now available (GenBank accession nos. AB161461 to AB161463[1]), and it appears that the protein fusion, which may increase the level of resistance, results from a mutation in the termination codon of the estX gene of Tn1825, leading to translational readthrough (Fig. 1A). Thus, sat2 is the only known cassette-associated streptothricin resistance gene, and sat1 is an estX-sat2 fusion.

Recently, the first cassette of Tn1825 has been found in class 1 integrons in GenBank accession nos. AY090896 (2) and AB121039 (1) (see Fig. 1B) but has been recorded as a sat cassette. In fact, the predicted 280-amino-acid polypeptide is not an acetyltransferase but is over 40% identical to several predicted proteins annotated as putative esterases or hydrolyses of the α/β fold superfamily (8). These are encoded in the genomes of Mesorhizobium loti, Bacillus cereus, Bacillus anthracis, and Bacillus thuringiensis and in a Sinorhizobium meliloti plasmid (Fig. 2). We have therefore named the cassette gene estX for esterase X. EstX is 42% identical to a protein encoded in multidrug resistance plasmids from Escherichia coli (7) and over 30% identical to RdmC, DauP, and DnrP from Streptomyces species. The latter are methyltransferases that catalyze one of the final “tailoring” reactions in the biosynthesis of rhodomycin, daunomycin, and daunorubicin, respectively (see reference 5). Whether EstX can inactivate any known antibiotic remains to be established.
Reports of the presence of a sat cassette should be treated with caution, unless the standard sat2 cassette (GenBank accession no. X15995) is cited (e.g., in reference 10) or an accompanying database entry is available for confirmation (e.g., accession no. X15995) is cited (e.g., in reference 10) or an additional homologue (not shown): DnrP (L40425), 94.6% identical to DauP; BA, product of BA2738 from \textit{Bacillus anthracis}; SMa, product of SMa1327 from \textit{Sinorhizobium meliloti}; and all other variants of EstX. The presence of the sat-2 cassette is, however, consistent with the presence of the sat2 cassette.

REFERENCES


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