

## Letters to the Editor

### Class 2 Integron with a Novel Cassette Array in a *Burkholderia cenocepacia* Isolate

*Burkholderia cepacia* complex (BCC) organisms are gram-negative opportunistic emerging pathogens associated with a poor prognosis for patients with cystic fibrosis (CF) (10). Carbapenems and ceftazidime are administered to patients suffering from BCC infections, and trimethoprim-sulfamethoxazole has historically been the drug of choice (6). Recently, the acquisition of resistance determinants to sulfamethoxazole located in the 3' conserved region of class 1 integrons in BCC isolates from CF patients has been described (2).

The goal of this study was to determine the presence of class 1, 2, and 3 integrons in a *Burkholderia cenocepacia* strain (BC1) isolated from the sputum of a 14-year-old CF patient in a surgery and transplant center in Buenos Aires, Argentina.

The phenotypic analysis was performed using biochemical reactions (4) and led us to identify the isolate as BCC. The genotypic identification by the *recA* PCR-restriction fragment length polymorphism method (9) resulted in a IIB *recA* lineage, corresponding to *Burkholderia cenocepacia*. A search for the presence of the cable pilin subunit gene (*cblA*) and the *B. cepacia* epidemic strain marker, by PCR with specific primers (9), yielded negative results. The BC1 strain was susceptible to trimethoprim-sulfamethoxazole (MIC = 1 µg/ml), meropenem (MIC = 0.012 µg/ml), ceftazidime (MIC = 0.19 µg/ml), and minocycline (MIC = 1 µg/ml) according to CLSI (formerly NCCLS) (1, 7).

By analysis of the class 1, 2, and 3 integrons present in the

BC1 strain by PCR with specific primers (Table 1), we found only a class 2 integron (8). In order to identify the inserted gene cassettes, PCR cartography was performed with primers described in Table 1. Two PCR products, obtained with the 125'CS and satR primers and the Inti2R and satR primers (Fig. 1) were sequenced on both strands using an ABI 373 sequencer and analyzed using the Genetics Computer Group (GCG) software (Wisconsin Package, version 10.3). Only a *sat2* gene cassette was found in the variable region, and it is noteworthy that this novel rearrangement did not have the *orfX* cassette always present in Tn7-like transposons (Fig. 1). Also, the analysis of the sequence led us to determine that the internal stop codon in the class 2 integrase gene was present as it has been previously described (3). Therefore, this novel rearrangement could likely have arisen from the action of a class 1 integrase from another element in *trans* (3). We also detected the presence of *tnsE* and *tnsD* genes located in the 3' conserved sequence region and involved in Tn7 transposition (11) (Table 1 and Fig. 1). We have named it Tn7::In2-1 (accession number DQ082896) in order to identify novel rearrangements of class 2 integrons in a simple manner.

Our results provide evidence that class 2 integrons located in the Tn7 family of transposons are able to be incorporated in the genome of BCC, as described for class 1 integrons recently (2).

TABLE 1. Primers used in this study

Primer	Sequence (5'-3')	Reference or source
Inti1R	TTC GAA TGT CGT AAC CGC	8
Inti1F	CGC GGC ATA GAC TGT AC	8
Inti2R	ACA CGC TTG CTA ACG ATG	8
Inti2F	GCA AAT GAA GTG CAA CGC	8
Inti3F	AGG TGC CTC CGG CAG CG	8
Inti3R2	GAC CAT GCT CGC CAC CGC CGA GA	This study
125'CS	TTT TTG TGC TGC CAT ATC CGT G	This study
23'CS	TGG GCT GAG AGA GTG GT	This study
satF	TGA GCA GGT GGC GGA AAC	This study
satR	TCA TCC TGT GCT CCC GAG	This study
dhfR1	AGC TGT TCA CCT TTG GC	5
dhfR1R	CCT GAA ATC CCC AGC AA	This study
aadA1	TCG ATG ACG CCA ACT AC	5
tnsER	TCG ATT TGC TGC TTT TGA TG	This study
tnsEFF	TTG CTC TCT AAC CAC TCT	This study
tnsDR	CCG TCT AAT TTG ATA ATC TTC	This study
tnsDF	GGG ATT GTT AGT CCT AAG C	This study

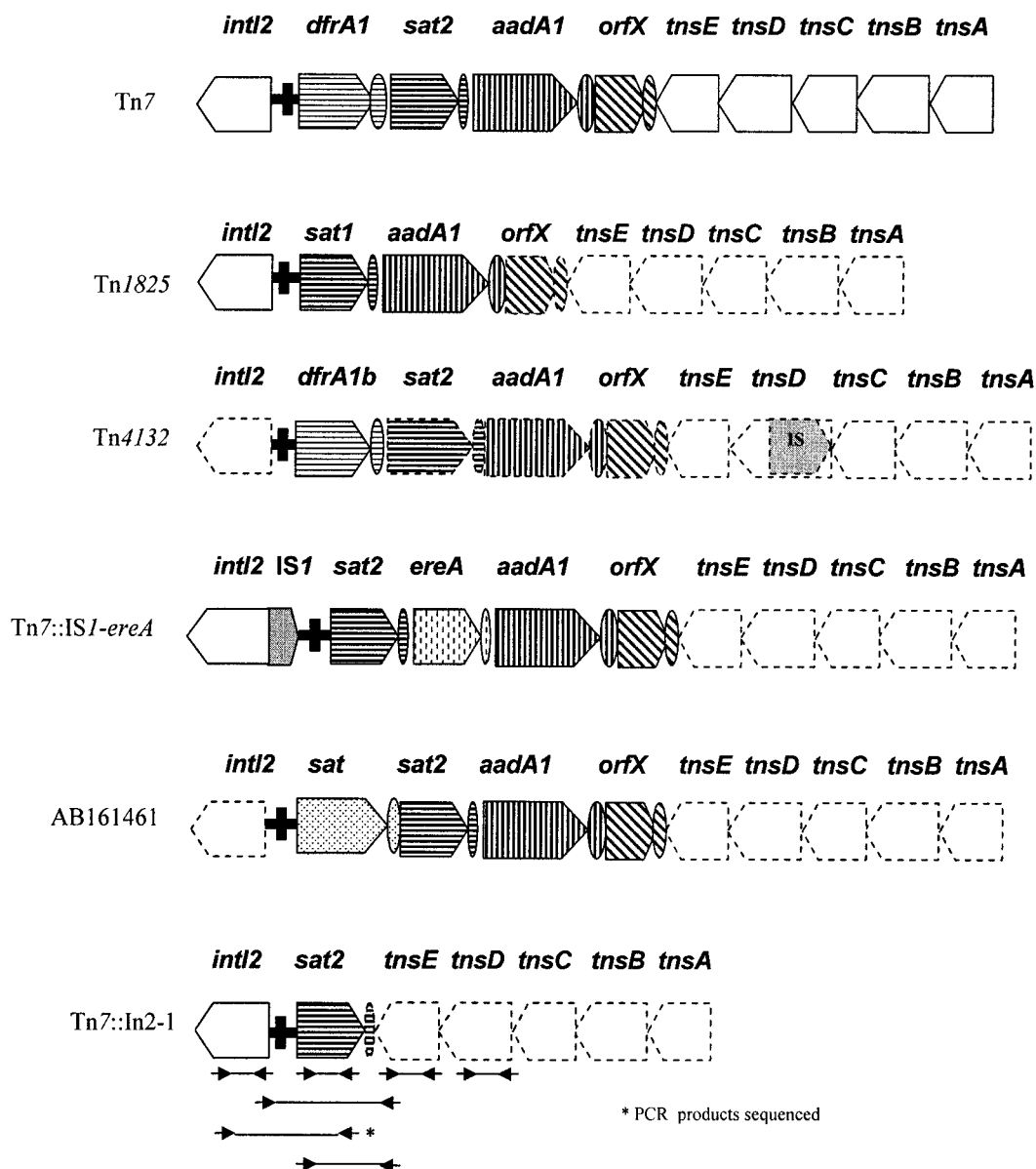


FIG. 1. Variable region of the class 2 integrons of the Tn7 family described in the literature, Tn7 (accession number NC\_002525), Tn1825 (X56815), Tn4132 (Z50804), Tn7::IS1-*ereA* (AY183453), a recently published class 2 integron with accession number AB161461, and Tn7::In2-1 from this study. The thin vertical open bar represents the *attI2* site, the ovals represent the *attC* sites of the gene cassettes, and dashed lines mean that the sequence is not available. All *intl2* genes sequenced reveal an internal stop codon (3).

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