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Recovery of a Functional Class 2 Integron from an *Escherichia coli* Strain Mediating a Urinary Tract Infection▽

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A class 2 integron was found in an *Escherichia coli* isolate mediating a urinary tract infection. Unlike other class 2 integrons from pathogens, the encoded IntI2 protein was functional. The integron possessed a *dfrA14* cassette, and a second novel cassette in which a lipoprotein signal peptidase gene is predicted.

Mobilized integrons are substantial contributors to the spread of antibiotic resistance genes. The three classes of integron that mostly contribute to the problem of multidrug resistance are classes 1, 2, and 3 (1, 13, 14), where classes are determined based on sequence differences in the respective IntI proteins (5). Of the three, class 1 integrons are the most abundant and are found in a diverse range of other mobile elements (12, 17), such as transposons and plasmids. However, class 2 integrons are also found in 4 to 20% of uropathogenic *Escherichia coli* strains (23, 24) as well as in other human pathogens (22), other animal pathogens (16), and various commensal bacteria (2, 4). In all of these cases, though, where examined, the *intI2* gene is inactive by virtue of possessing a premature in-frame stop codon. Interestingly, however, in one study class 2 integrons were identified in commensal bacteria from bovine fecal material and hides. Here, the normally present stop codon is replaced with a glutamine codon and it is therefore likely that the associated IntI2 protein is functional, although this has not been demonstrated experimentally (3). Such putatively functional integrons have not been seen in either human commensals or pathogenic bacteria. Clearly, if they were to be found in such bacteria they would represent an additional mechanism by which resistance gene cassettes could be mobilized in pathogens.

In a survey of strains mediating a urinary tract infection from different individuals in Uruguay (19), 15 of 104 strains were identified that possessed a class 2 integron on the basis of generating a 789-bp PCR product with an *intI2*-specific primer pair (21). All 15 PCR products were sequenced, and 13 possessed the internal stop codon, confirming that the nonfunctional version of *intI2* predominates in this population. However, two *E. coli* strains, designated 3843 and 8157, from separate individuals generated a sequence that implied a functional *intI2* gene was present. Consequently, a genomic fosmid library was constructed from strain 8157 and the class 2 integron sequence was determined. The *intI2* gene was different from the corresponding gene in Tn*7* (accession no. AJ001816) at six positions, including in the stop codon (TAA) in Tn*7*, which in strain 8157 was the glutamine codon CAA.

This is the first report of a potentially functional class 2 integron from a human pathogen. To confirm that the *intI2* gene from *E. coli* isolate 8157 encoded a functional integrase, an amplicon including the gene was generated and cloned into pUC19 downstream of the P_{lac} promoter. The recombinant plasmid was used to measure the integration frequency of the *aadB* cassette *attC* site into a target class 1 integron, as described previously (7, 11). The assay used measures the ability of an integron integrase to catalyze an integrative recombination reaction between two sites: normally either *attC* versus *attI* or *attC* versus *attC*. It was found that IntI2 could efficiently catalyze an integrative recombination reaction (Table 1). The point of insertion in the target conjugative plasmid, pMAQ495 (7), which contains a class 1 integron, was determined by PCR mapping (15). It was found that for 10/10 (two from five independent assays) cointegrate junctions, insertion had occurred at the pMAQ495 *orfA attC* site. This is consistent with observations that integron integrases do not efficiently recognize heterologous *attI* sites (8) and that *orfA* is the preferred *attC* target when *attI1* is not favored (9).

To determine whether the class 2 integron was on a transferable plasmid, the strain 8157—which is sulfamethoxazole (SMX) and chloramphenicol (CHL) resistant and nalidixic acid (NAL) and rifampin (RIF) sensitive—was mated (20) with the *E. coli* strain Top10 (SMX and CHL sensitive and NAL and RIF resistant). Transconjugants appeared after selection on media containing SMX and NAL at a frequency of 1.0×10^{-4}/recipient. A total of 24 screened transconjugants were also resistant to CHL and RIF, 3 of which were screened by PCR and were found to be positive for the *intI2* gene. Plasmid typing of the transconjugants by Inc/rep multiplex PCR (6) revealed a single amplicon consistent with the presence of an IncP plasmid.

In total, about 4,500 bp of DNA sequence was determined. The class 2 integron possessed a two-cassette array. The first of

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TABLE 1. Frequency of IntI2-mediated recombination

Integrase	Test element[a]	Range of recombination frequencies	Avg. cointegration frequency (no. of independent assays)
IntI1	*aadB attC*	4.5×10^{-3}–1.6×10^{-2}	1.1×10^{-2} (5)[c]
IntI2[b]	*aadB attC*	3.2×10^{-5}–3.6×10^{-4}	1.5×10^{-4} (7)
IntI2	None	5.3×10^{-8}–6.0×10^{-7}	4.1×10^{-7} (3)

[a] The *aadB attC* site was cloned into pMAQ28 (11), and the no-element control was pACYC184.
[b] This protein is encoded within the plasmid designated pMAQ1047.
[c] Value as measured by Collis et al. (9).

these was the *dfrA14* gene cassette seen commonly in class 1 integrons (10, 18). The second cassette included a novel open reading frame (ORF) not previously associated with cassettes and predicted a protein that matches a family of lipoprotein signal peptidases. The best match (4e−31; accession no. YP_001230301) was to a protein from *Geobacter uraniireducens*. Beyond this second cassette was another ORF that predicted a putative outer membrane lipoprotein (best match, 1e−23 to YP_411052) from *Nitrosospira multiformis*. This ORF was not obviously in a gene cassette in that an *attC* site could not be identified. Also, the putative outer membrane lipoprotein gene associated with the class 2 integron in strain 8157 is not functional as it has a stop codon at position 40 in an unprocessed predicted protein that would otherwise be 117 amino acids in length.

Class 2 integrons are found in a significant proportion of multiresistant human isolates, although the associated IntI2 protein, where examined, is nonfunctional (23, 24). Here we show that a functional class 2 integron has appeared in a pathogenic *E. coli* strain and can autonomously acquire new cassettes and is apparently carried on an IncP plasmid that can transfer at high frequency. It will be important to determine whether this integron appears in different contexts, both geographical and genetic, beyond the single observation made here. This integron also displays evidence of acquiring new types of genes relevant to pathogens not obviously present in mobilized integrons previously. It will be interesting to investigate the context of this functional class 2 integron further to determine whether it is associated with Tn7 or some other transposon since such an association would provide another mechanism of mobilization.

Nucleotide sequence accession number. The sequence of the strain 8157 class 2 integron and adjacent sequence have been submitted to GenBank under accession no. EU780012.

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