ABSTRACT

Four *Salmonella typhi* isolates (S2, S6, S8, and S9), each harbouring a large conjugative R plasmid that confers resistances to ampicillin (Ac^r), chloramphenicol (Cm^r), cotrimoxazole (Ctm^r), streptomycin (Sm^r), and tetracycline (Tc^r), were investigated for antibiotic resistance transposons. Transposition experiments were performed with pUB307, a conjugative R plasmid conferring resistances to kanamycin (Km^r) and Tc^r, as the recipient replicon. Two major antibiotic resistant transconjugants were obtained, $Ac^rCtm^rKm^rSm^rTc^r$ and $Ac^rCm^rCtm^rKm^rSm^rTc^r$. The results showed that the transposons isolated from the four *S. typhi* isolates were similar despite their different origins.

Results of the transposition, transformation, and secondary transposition experiments indicated the existence of two multiple antibiotic resistance transposons. One encoded $Ac^rCtm^rSm^r$ and the other, Cm^r and probably also Tc^r. On the R plasmid of *S. typhi*, another copy of the $Ac^rCtm^rSm^r$ transposon may be associated with the Cm^r transposon and it could transpose independently of the Cm^r transposon into pUB307. The insertion of the Cm^r transposon on pUB307 was unstable and the Cm^r transposon often transposed again onto the *Escherichia coli* chromosome by a conservative or 'cut and paste' mode of transposition.

An $Ac^rCtm^rSm^r$ transposon derived from *S. typhi* S8 was inserted into a recombinant pUB307, pCL8, and it appeared to be identical to Tn*5410,* previously detected in S8 by Kadambeswaran (1993). In a shotgun cloning experiment, DNA fragments of *Sal*I-digested pCL8 were cloned into plasmid vector pKan in *E. coli* DH5α. Recombinant pKan that carried 2.5 kb Ac^r and 5.5 kb Sm^r inserts were designated as pCLA25 and pCLS55, respectively. A Ctm^r transformant that harboured a recombinant pUB307, pCLC600, was also obtained. pCLC600 resulted from recircularization of *Sal*I-digested pCL8. DNA fragments of *Sau*3AI partially-digested pCLC600 were cloned into *Bam*HI-digested pKan in *E. coli* DH5α. Five Ctm^r transformants were obtained. One of them harboured a recombinant pKan, pCLC2, that carried a 4.3 kb insert.

Southern hybridization confirmed that the 2.5 kb Ac^r *Sal*I-insert in pCLA25 originated from the R plasmid pST8 of *S. typhi* S8. The 2.5 kb Ac^r insert was subcloned into a bacteriophage vector M13mp19 in *E. coli* DH5αF'. An Ac^r transfectant, harbouring M13mp19-Ac^r(2.5), was obtained and its RF DNA was purified for nested deletions by using a nested deletion kit. After unidirectional nested deletions and transfection of the nested deleted M13mp19-Ac^r(2.5) DNA into *E. coli* DH5αF', a library comprising a series of progressively deleted clones was obtained. The smallest recombinant M13mp19 RF DNA isolated from this library that still conferred Ac^r carried a 1.8 kb insert and was designated as M13mp19-Ac^r(1.8). The 1.8 kb insert of M13mp19-Ac^r(1.8) was subcloned into bacteriophage vector M13mp18 in *E. coli* DH5αF'. An Ac^r transfectant, harbouring M13mp18-Ac^r(1.8), was obtained and its RF DNA was purified for construction of a second nested deletion library.

Deleted M13 derivatives from both nested deletion libraries were used to determine the nucleotide sequences of both strands by manual and automated dideoxy ribonucleotide chain termination sequencing. Analysis of the 1814 nucleotides sequenced showed the presence of three distinct segments. One segment of 1444 nucleotides was identical to the right section of transposon Tn*2*, extending from the middle of the *tnpR* resolvase gene to the right inverted repeat (IR) end, including the Ac^r TEM-1 β-lactamase gene. The *tnpR* resolvase gene was disrupted by a second segment comprising a 351 nucleotide sequence common to the IS*26*R element and its variants. The IS*26*-like segment extended from its left IR end to its unique *Sal*I site. A third segment of 19 nucleotides flanked the IR end of the Tn*2*-like segment and the 19 nucleotides were identical to that of those flanking the *sulII strA strB* (Su^rSm^r) gene cluster on plasmid RSF1010.

Results from this study and circumstantial evidence from other workers suggest that Tn*5410* was a Tn*5086*-like element from the Tn*21* family and might contain three inserted copies of IS*26*-like elements. A genetic structure of Tn*5410* was proposed.