CHAPTER FOUR DISCUSSION AND CONCLUSION

4.1 Detection of $Ac^rCtm^rSm^r$ transposon and Cm^r transposon

From the results in Sections 3.1 to 3.3, it is concluded that two types of antibiotic resistance transposons were detected in the R plasmids of *S. typhi* S2, S6, S8, and S9. One encoded $Ac^rCtm^rSm^r$ and the other at least Cm^r and probably Tc^r too. The profiles generated from restriction endonuclease digested recombinant pUB307 indicated that each type of these transposons from pST2, pST6, pST8, and pST9 was very similar if not identical to one another. These transposons may or may not be present on the chromosome of *S. typhi* S2, S6, S8, and S9.

The transposition frequency of the Cm^r transposon was distinctly lower than that of the $Ac^rCtm^rSm^r$ transposon. These results suggested that there were two different transposons on the R plasmids of *S. typhi*. These two transposons (i.e., the $Ac^rCtm^rSm^r$ transposon and the Cm^r transposon) would not likely to transpose independently and simultaneously when transconjugants were selected on one antibiotic. The probability for the two transposons to transpose independently and simultaneously onto the same replicon would be below the detection limit of 10^{-9} used in the present transposition experiments. Therefore, the $Ac^rCtm^rSm^r$ transposon and the Cm^r transposon may be physically linked on the R plasmid of *S. typhi*. The results of all transposition experiments indicated that the Cm^r transposon most likely also carried another copy of the $Ac^rCtm^rSm^r$ transposon. This is because the $Ac^rCtm^rSm^r$ transposon was able to transpose independent of the Cm^r transposon but not vice versa; Cm^s transconjugants were usually generated when the transconjugants were selected for Ac^r or Ctm^r but Ac^rCtm^r transconjugants were usually generated when

transconjugants were selected for Cm^r.

When Cm^r transposed from the *S. typhi* plasmid to pUB307, it probably carried the $Ac^rCtm^rSm^r$ transposon along. Therefore, almost all the transconjugants obtained were Ac^r and Ctm^r when they were selected for Cm^r. However, the $Ac^rCtm^rSm^r$ transposon, carried by the Cm^r transposon on the *S. typhi* plasmid, could transpose to pUB307 independently and at a higher frequency than the Cm^r transposon. Consequently, most transconjugants were $Ac^rCm^sCtm^rKm^rSm^rTc^r$ when they were selected for Ac^r or Ctm^r.

Recombinant plasmids from $Ac^rCm^rCtm^rKm^rSm^rTc^r$ transconjugants generated mostly $Ac^rCm^sCtm^rKm^rSm^rTc^r$ transformants, indicating that these Cm^r transconjugants harboured recombinant plasmids that carried the $Ac^rCtm^rSm^r$ transposon but not the Cm^r transposon.

The Cm^r transposon that transposed from the *S. typhi* plasmids to pUB307 appeared to be unstable on recombinant pUB307 and preferred to transpose to *E. coli* chromosome. The tendency of the Cm^r transposon to transpose to the chromosome could be due to the presence of many insertion sites on the chromosome. The transposon Tn*7* is known to have such a site which is designated as *att* Tn*7* (Lichenstein and Brenner, 1982). The Cm^r transposon, carrying another copy of the $Ac^rCtm^rSm^r$ transposon, could have transposed from recombinant pUB307 to the chromosome of *E.coli* UB1637 via a conservative or 'cut and paste' mode of transposition (Fig. 54) (Berg and Berg, 1987; Sherratt, 1989). Subsequently, the $Ac^rCtm^rSm^r$ transposon carried by the Cm^r transposon that had transposed onto the chromosome, might transpose back to pUB307, independent of the Cm^r transposon. This transposition event probably

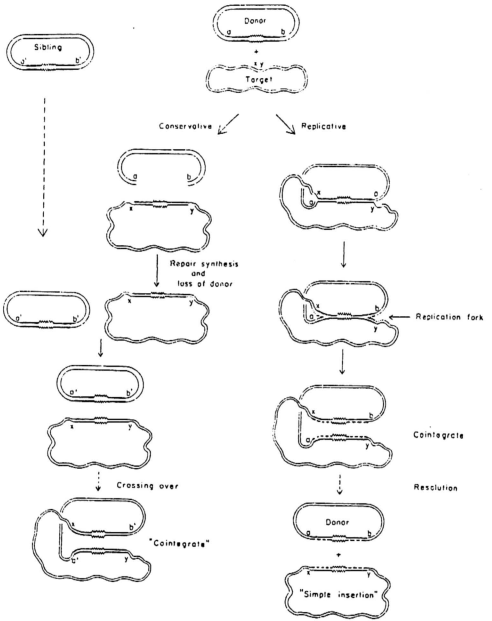

Figure 54 : Formation of cointegrates and of simple insertions by both conservative and replicative transposition (Sherratt, 1989)

occurred via a replicative mode of transposition because the cured derivatives of $Ac^rCm^rCtm^rKm^rSm^rTc^r$ were $Ac^rCm^rCtm^rKm^sSm^rTc^r$. However, the $Ac^rCtm^rSm^r$ transposon occasionally transposed via a 'cut and paste' mode, resulting in a loss of the $Ac^rCtm^rSm^r$ phenotype from the Cm^r transposon on the chromosome. Consequently, an $Ac^sCm^rCtm^sKm^rSm^sTc^r$ transconjugant was obtained (Table 9). The use of both the replicative and 'cut and paste' modes of transposition have been observed in Tn*3*-like transposons (Sherratt, 1989) and Tn*7* (May and Craig, 1996).

The transposition frequencies of the two types of transposons, particularly the Cm^r transposon, were reduced when *E.coli* UB5201 harbouring the *S. typhi* plasmid and pUB307 was kept on Dorset egg slant for more than two weeks. The prolonged incubation could have allowed the Cm^r transposon on the *S. typhi* plasmid to transpose preferentially into the chromosome of UB5201, instead of transposing onto pUB307. Therefore, a shorter incubation time may favour the detection and isolation of the Cm^r transposon.

4.2 Cloning of antibiotic resistance genes of pCL8

Previously, Kadambeswaran (1993) had detected an $Ac^rCtm^rSm^r$ transposon from *S. typhi* S8. This transposon was subsequently designated as Tn*5410* (Professor Koh C.L., pers. comm.). In this study, an $Ac^rCtm^rSm^r$ transposon with similar transposition characteristics was also isolated from *S. typhi* S8. Therefore, it was assumed to be identical to Tn*5410*.

Based on the restriction mapping in Section 3.4.1, a copy of Tn*5410* was deduced to be located in between the *SalI* and *SmaI* sites within the *tetA* gene (Waters *et al.*, 1983) of a recombinant pUB307, designated as pCL8, isolated from an

$Ac^rCtm^rKm^rSm^rTc^s$ transconjugant. A 2.5 kb Ac^r *Sal*I fragment and a 5.5 kb Sm^r *Sal*I fragment were separately cloned into the plasmid pKan. Plasmid pKan (Micklos and Freyer, 1990) was used because it contains a unique *Sal*I site and other plasmid vectors that do not carry Ac^r, Ctm^r, or Sm^r were not available to me. The *Sal*I-digested fragments of pCL8 were used for cloning because digestion of pUB307 with *Sal*I generated only two large fragments. Furthermore, one of these *Sal*I sites flanks Tn*5410* in pCL8.

Among the five Ctm^r clones obtained from the shotgun cloning performed on pCLC600, a clone harbouring pCLC2 that carried the smallest insert of 4.3 kb was obtained. It was fortunate that the *Bam*HI-site proximal to the Km^r gene of the pKan vector was intact even after ligation with *Sau*3AI-digested fragments of pCLC600. In the process of constructing pKan from pUC19, Micklos and Freyer (1990) removed the restriction sites between *Bam*HI and *Hin*dIII from the multiple cloning sites of pUC19 but the restriction sites between *Eco*RI and *Bam*HI sites in the multiple cloning sites were retained. Therefore, the 4.3 kb insert of pCLC2 could be excised with an *Eco*RI-*Bam*HI double digestion and subcloned easily into a better vector such as pUC19.

Among all the antibiotic resistance fragments cloned, the 2.5 kb Ac^r *Sal*I insert in the recombinant pKan, pCLA25, was the smallest. Therefore, it was subjected to further characterization.

4.3 Southern hybridization

Southern hybridization was performed to confirm that the 2.5 kb Ac^r *Sal*I-insert of pCLA25 originated from pST8 of *S. typhi* S8. It should be noted that even though pST8 and other related large R plasmids were isolated by Kadambeswaran (1993) and

Chong (1992), pST8 could not be isolated by the methods reported (Birnboim, 1983; Kado and Liu, 1981). Instead pST8 was isolated by a modified method of Hansen and Olsen (1978). This method appeared to be reliable for the isolation of pST8.

Results from the hybridization confirmed that the 2.5 kb Ac^r *Sal*I-insert was present in pST8. The labelled 2.5 kb Ac^r probe also hybridized to the 1.6 kb fragment of the 1 kb ladder and the 4.2 kb *Sal*I-digested pKan. The 1.6 kb fragment of 1 kb ladder is a *Hpa*I-fragment of pBR322 and it contains the Ac^r gene of pBR322 (Sutcliffe, 1978; Hartley and Donelson, 1980). Strong signal also appeared on the pKan vector band because it contains a partially deleted Ac^r gene derived from pUC19 (Micklos and Freyer, 1990).

The faint signal detected on the 5.5 kb Sm^r *Sal*I-fragment of pCL8 indicates that this fragment may have some similarity to the 2.5 kb Ac^r probe. However, no signal was detected at the 5.5 kb region of *Sal*I-digested pST8. This may be due to the low copy of the target fragment and the lack of similarity between the probe and the target.

4.4 Partial sequences of Tn*5410*

4.4.1 The Tn*2*-like segment

Sequence similarity between the Tn*2*-like segment and Tn*2* occurs between a stretch of 1444 nucleotides extending from the middle of *tnpR* resolvase gene to the right inverted repeat (IR) of Tn*2*. The Ac^r gene in this segment encodes the TEM-1 β-lactamase. This β-lactamase is identical to that found in Tn*3*, despite three silent nucleotide substitutions (Chen and Clowes, 1987).

Sequence variations among Tn*1*, Tn*2*, and Tn*3* have been compiled by Chen and Clowes (1987) and Goussard and Courvalin (1991), and the nucleotide positions of Tn*1*

and Tn*2* are assigned according to the nucleotide positions of Tn*3*. By comparison, the Ac^r gene in Tn*1* differs from that of Tn*3* by five nucleotide substitutions, in which four are silent mutations. Base substitution from C to A at position 4058 results in the substitution of Gln in Tn*3* to Lys in Tn*1*. This difference accounts for the isoelectric focusing difference observed between the two β-lactamases from Tn*1* and Tn*3* (Matthew *et al.*, 1979). As a consequence, the β-lactamase from Tn*1* is designated the TEM-2-type (Matthew *et al.*, 1979). The low divergence of 0.55 for the Ac^r gene of Tn*1*, Tn*2* and Tn*3* suggests that this segment only diverged relatively recently (Chen and Clowes, 1987).

There is a single difference in the non-coding region of Tn*2*-like segment sequenced at position 754, i.e., a nucleotide G, instead of an A at position 3916 in Tn*3* (Heffron *et al.*, 1979) (Fig. 50a). This mutation is located near the promoter for β-lactamase transcription. Tn*2* has only one β-lactamase transcription initiation site, which is identical to the P3 promoter of Tn*3* (Brosius *et al.*, 1982; Chen and Clowes, 1987). In Tn*1*, base substitution of T at position 3773 in place of a C in Tn*3* results in the creation of two stronger overlapping promoters, in which the -10 box of the upstream Pa promoter overlaps with the extended -35 box (Hawley and McClure, 1983) of the downstream Pb promoter (Chen and Clowes, 1984).

Transposons Tn*2* and Tn*3* differ by a total of 22 bases, and 19 of them are clustered in the *tnpR* gene segment, whereas Tn*1* and Tn*3* differ by only three bases (Chen and Clowes, 1987). Based on the higher nucleotide divergence between the *tnpR* genes of Tn*2* and Tn*3* (or Tn*1*) compared to that of the Ac^r genes and the noncoding sequences in these transposons, Chen and Clowes (1987) proposed that the *tnpR*

segment of Tn*2* was derived from an earlier progenitor than the similar segments of Tn*3* and Tn*1*. In this study, only one silent mutation was observed in each of the non-coding and *tnpR* regions sequenced, when compared to similar regions of Tn*3*. The nucleotide differences between the *tnpR* gene of Tn*2* and Tn*3* only occur at the third coding base of the codons. Therefore, no amino acid sequence changes resulted.

Truncated Tn*3*-like elements encoding Ac^r have been reported on numerous plasmids. Fayet *et al.* (1982) found a plasmid that contains a 1.6 kb segment homologous to the Ac^r gene region of Tn*2301*, a Tn*3*-like transposon. This 1.6 kb DNA segment is not transposable, but when linked to the *tnpA* part of Tn*2301* by ligation, it becomes a functional transposon. Plasmid pFM739, an RSF1010-like R plasmid from *Neisseria sicca*, also contains a truncated Tn*3*-like element that is transposition defective (Rotger *et al.*, 1986). The plasmid pBP749 was reported to carry a 1.18 kb segment that is homologous to Tn*3* (Gerlach and Wiedemann, 1985). A segment that is 100% homologous to the Ac^r, *tnpR* and part of *tnpA* of Tn*1* was found in the multiple antibiotic resistance region of plasmid pCFF04 (Mabilat *et al.*, 1992).

One ended transposition had been reported for Tn*3* family transposon (Arthur *et al.*, 1984; Motsch and Schmidt, 1984; Heritage and Bennett, 1985). This process involves transposition-like events that occur in the presence of transposase with donor replicons that contain only one compatible IR sequence. However, one ended transposition for Tn*3* is about 1000 times less efficient than regular transposition (Ehrlich, 1989). Furthermore, the Tn*2*-like segment did not contain the sequences that encode the *tnpA* transposase. Therefore, it is unlikely that the integration of the Tn*2*-like segment into the progenitor of Tn*5410* was mediated by a one-ended

transposition process. It is also unlikely that this Tn*2*-like segment was inserted into the progenitor of Tn*5410* through a spontaneous transposition event that does not involve transposase that was recently reported by Rappleye and Roth (1997). Such event is extremely rare and currently, only one case was reported.

The presence of an IS*26*-like element flanking the truncated end of the Tn*2*-like segment suggests that the Tn*2*-like element could have transposed into the progenitor of Tn*5410* as an intact element and followed by deletion of the left segment of the Tn*2*-like element including the 38 bp IR sequence, *tnpA* gene and part of the *tnpR* gene that flanks the IS*26*-like segment sequenced. This hypothesis is supported by the presence of short sequence homology at the junction between the IR sequence of the IS*26*-like segment (adjacent to the Tn*2*-like segment) and Tn*3* (Fig. 53). Wrighton and Strike (1987) also found such homology between IR ends of IS*176* and the *tnpR* gene of Tn*1701* (Grindley and Nakada, 1981; Lambert *et al.*, 1987; Cannon and Strike, 1992). IS*176* is identical to IS*26* (Barg *et al.*, 1995) and Tn*1701* to Tn*2* (Yamada *et al.*, 1979). Tn*1701* was later designated as Tn*4353* by Cannon and Strike (1992).

Although the junction between the IS*26*-like segment and the Tn*2*-like segment found in this study differs from the one reported by Wrighton and Strike (1987), in this case, a higher number of identical nucleotides (i.e., 20 bases) was obtained (Fig. 53). An IS*26*-like element could have inserted within the *tnpR* gene and followed by a unidirectional deletion facilitated by sequence homology between the end of the IS*26*-like element and the putative deletion endpoint. This implies that the IS*26*-based transposase may have erroneously recognized the Tn*2* sequences as a terminus of the IS*26*-like element, which is its presumed site of action. Alternatively, this sequence may

have acted as a substrate for *rec*-dependent homologous recombination (Wrighton and Strike, 1987). The homologous recombination event could have occurred in the *S. typhi* host but not in the *recA E. coli* hosts used in the transposition experiments.

4.4.2 The IS*26*-like segment

The IS*26* element has been reported under several pseudonyms, such as IS*6* (Berg *et al.*, 1975), IS*15-delta* (Trieu-Cout and Courvalin, 1984), IS*46* (Brown *et al.*, 1984), IS*140* (Brau and Pieperberg, 1983), IS*160* (Nies *et al.*, 1985), and IS*176* (Wrighton and Strike, 1987). This element is 820 bp long and carries 14 bp perfect terminal inverted repeats.

Insertion sequence IS*15*, formerly designated as IS*1522* (Labigne-Roussel and Courvalin, 1983), is a close relative of IS*26*. It is formed by transposition of IS*15-delta* onto itself. The two IS elements that constitute IS*15* have been designated as IS*15-delta*I and IS*15-delta*II, which differ from IS*26* at only 3 and 1 point substitutions, respectively, and are considered to be genetic variants of IS*15-delta* (or IS*26*) (Table 12). Homologous recombination between IS*15-delta*I and IS*15-delta*II would lead to IS*26* (Trieu-Cout and Caurvalin, 1984). Two other variants of IS*26*, designated as IS*15-delta*III (Ouellette *et al.*, 1987) and IS*15-delta*IV (Nucken *et al.*, 1990), differ from IS*26* at only one point substitution each (Table 12). In this study, it is not known if the IS*26*-like element discovered is identical to IS*26* or its variants because the segment cloned and sequenced is common to IS*26* and its variants.

The promoter of the IS*26* transposase gene is located within an IR of IS*26* and is found in the IS*26*-like segment sequenced. This transposase belongs to a superfamily of transposases that carry a D, D(35)E protein motif (Hartl *et al.*,

Table 12: Comparison of the nucleotide sequences of IS*26* and its variants

Nucleotide position	**IS*26***	**IS*15 delta* I**	**IS*15 delta* II**	**IS*15 delta* III**	**IS*15 delta* IV**
459	G	A	G	G	A
563	A	A	A	G	A
613	G	A	G	G	G
614	G	A	G	G	G
764	T	T	C	C	T

Note: The nucleotide position is based on the sequence of IS*26*R (Mollet *et al.*, 1983)

1997). This tranposase is thought to be involved in deletion of sequences flanking IS26-like elements reported in several plasmids, e.g., NTP16 (Wrighton and Strike, 1987), R46 (Hall, 1987), and N3 (Brown *et al.,* 1984).

IS*26*-like elements contain unique *Pst*I and *Sal*I sites at position 159 and 252, respectively (Mollet *et al.*, 1983). These two sites were found on the IS*26*-like segment sequenced. For ease of explanation, henceforth the IS*26*-like segment sequenced, which extends from the *Sal*I site to the IR end, will be designated as the left segment IS*26*R (Fig. 55). The 'R' here refers to the IS*26* that is located on the right end of the composite transposon Tn*2680* (Mollet *et al.*, 1983).

Besides the Km^r transposon Tn*2680*, which is identical to Tn*6* (Berg *et al.*, 1975), other IS*26*-based elements are also found in other Km^r composite transposons, such as Tn*1525* (Trieu-Cout and Courvalin, 1984) and Tn*4352* (Wrighton and Strike, 1987). IS*26*-based composite transposons carrying other known antibiotic resistance genes include the Ac^{r}Gen^r Tn*2922* (Martin *et al.,* 1987) and the Tp^r Tn*5091* (Sundstrom *et al.*, 1995) and its unnamed Tp^r relative (Barg *et al.*, 1995), are flanked by IS*176*, i.e., IS*26*. IS*26* has relatives that are similar but not identical. One of these relatives is the IS*6100* on a Su^r transposon of *Mycobacterium fortuitum* (Martin *et al.*, 1990).

Interestingly, in all IS*26*-based composite transposons reported above, the IS*26*-like elements are arranged in direct repeats. When three copies of IS*26* are found flanking multiple antibiotics resistance determinants, the second copy is in the same orientation with either the first or third copy but not both (Iida *et al.*, 1982; Allard *et al.,* 1993; Kim and Aoki, 1994).

Directly repeated copies of IS*26* are thought to be essential for the transposition

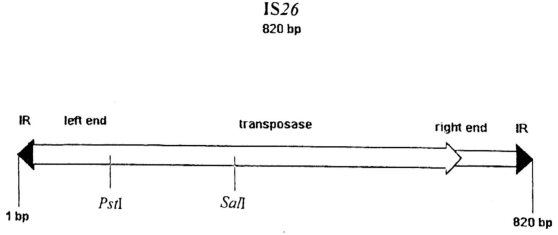

Figure 55 : The structure of IS*26*

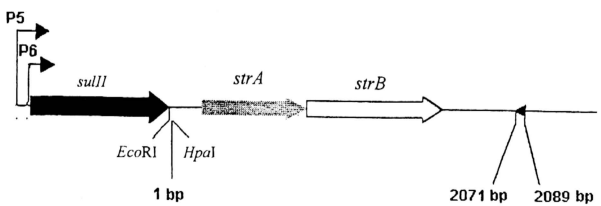

Figure 56 : The *sulII strA strB* gene cluster of RSF1010

of IS*26*-based composite transposon. IS*26* elements require a cointegrate formation step that involves one copy of the IS*26* and a resolution step that involves *recA* dependent homologous recombination between the directly repeated copies (Galas and Chandler, 1989). Therefore, IS*26*-based composite transposons are 'pseudo-composite transposon' because their transposition involves homologous recombination.

4.4.3 The RSF1010-like segment

Although the RSF1010-like segment sequenced consisted of only 19 bp, it provides useful information about the 5.5 kb Sm^r *Sal*I-fragment that is adjacent to the 2.5 kb Ac^r *Sal*I-fragment in pCL8. The location of the RSF1010-like segment, which is between the Tn*2*-like segment and the adjacent 5.5 kb Sm^r *Sal*I fragment, and its orientation suggest that the Sm^r gene or genes in the 5.5 kb Sm^r *Sal*I fragment are similar to the Su^r (*sulII*) Sm^r (*strA strB*) gene cluster of RSF1010 (Fig. 56) (Scholz *et al.*, 1989).

Theoretically, the distance from RSF1010-like segment on the 2.5 kb Ac^r fragment to the *Sal*I site and from the *Sal*I site to the 5.5 kb Sm^r fragment is sufficient to accommodate a copy of IS*26*-like element inserted in either direct or inverse orientation with respect to the copy of IS*26*-like element on the other terminus of the 2.5 kb Ac^r fragment.

Based on the above assumption that the Sm^r determinants in Tn*5410* are similar to those of RSF1010, Miss Rameswari Pararajasingam Pillai (pers. comm.), a Master of Biotechnology student from this laboratory, subcloned a 2.6 kb Sm^r *Sal*I-*Eco*RI fragment from the 5.5 kb Sm^r insert of pCLS55. As expected, the Sm^r determinant appeared to be promoterless as in RSF1010 and she found that pCLS55 also conferred resistance to Su, as in the *sulII strA strB* cluster of RF1010 (Scholz *et al.*, 1989). In

RSF1010, expression of the entire cluster of *sulII strA strB* is driven by the P_5 and P_6 promoters located at the 5' end of the *sulII* gene.

Miss R.P. Pillai also found that the characteristic *Pst*I site of IS*26*R (Fig. 55) was absent near the *Sal*I-terminus of the 2.6 kb Sm^r *Sal*I-*Eco*RI fragment that she subcloned. We may deduce that if a copy of IS*26*-like element is inserted in the junction between the 2.5 kb Ac^r and 5.5 kb Sm^r fragments, then this copy should be inserted in the inverse orientation to the IS*26*-like element (designated as IS*26*-1) on the other terminus of the 2.5 kb Ac^r fragment. Although nucleotide sequences beyond the RSF1010-like segment have not been determined, the putative second copy of the IS*26*-like element (designated as IS*26*-2) is assumed to be inserted in the reverse orientation to the first, in the following discussion.

Based on this assumption, the putative IS*26*-like segment on the 5.5 kb Sm^r fragment adjacent to the 2.5 kb Ac^r fragment may be a left segment of an IS*26*R-like element and therefore, could not have generated the weak signal at the 5.5 kb region found on the X-ray film obtained in the Southern hybridization (Fig. 42b, lane 4). This is because the labelled 2.5 kb Ac^r fragment probe contained the right segment of IS*26*R. Therefore, a third copy of IS*26*-like element (designated as IS*26*-3) may be present on the other terminus of the 5.5 kb Sm^r fragment and is in direct orientation to the second copy.

4.4.4 The structure of Tn*5410*

Recently, Lye (1997) has subcloned a 2.2 kb Tp^r *Hin*dIII-*Bam*HI fragment from the 4.3 kb Ctm^r insert of pCLC2. Partial sequencing of this fragment from both termini showed that nucleotide sequences at the *Bam*HI terminus are almost identical to the

integrase (*int*) gene of the integron of Tn*5086* and Tn*21* (Sundstrom *et al.*, 1993) and the nucleotide sequences at the *Hin*dIII terminus are almost identical to part of the Tp^r (*dhfrVIII*) and *qacEΔI* gene cassettes of Tn*5086*. The *int* genes in Tn*5086* and Tn*21* are almost identical except for a group of three consecutive G's close to the start of the *int* gene in Tn*21* which is missing in Tn*5086* (Mercier *et al.*, 1990; Sundstrom *et al.*, 1993). The *qacEΔI* gene cassette is part of the conserved region of *sulI* associated integron (Stokes and Hall, 1989; Paulsen *et al.*, 1993).

These findings suggest that Tn*5410* may be closely related to the Tn*21*-like transposon, Tn*5086* (Fig. 57a). Transposon Tn*5086*, like most Tn*21*-like transposons, confers resistance to $HgCl_2$. However, when *S. typhi* S8 was tested on an LB plate containing $HgCl_2$ (50 μg/ml), no growth was observed. Therefore, it is possible that the putative third copy of IS*26*-like element (designated as IS*26*-3) may have inserted into the mercury resistance operon of a Tn*5086*-like progenitor of Tn*5410*. A genetic organization of Tn*5410* is proposed here (Fig. 57b).

The 6.7 kb *Sal*I fragment of pCL8, adjacent to the 5.5 kb Sm^r fragment, is yet to be characterized. It may contain the putative mercury resistance operon of the Tn*5086*-like element.

The Cm^r transposon has not been isolated yet and the presence of Tc^r on this transposon has not been confirmed. A multiple antibiotic resistance transposon comprising a cluster of resistance genes, including Cm^r and Tc^r, interposed by multiple copies of IS*26*-like elements, i.e., (IS*26* or IS*15*)-Cm^r-IS*1*-Su^r-Sp^r-IS*26*-Tc^r-IS*26*-Ac^r-(IS*26* or IS*15*), have been reported in *S. ordonez* BM2000 by Allard *et al.* (1993). It would be interesting to investigate if a similar association also occurs between Tn*5410*

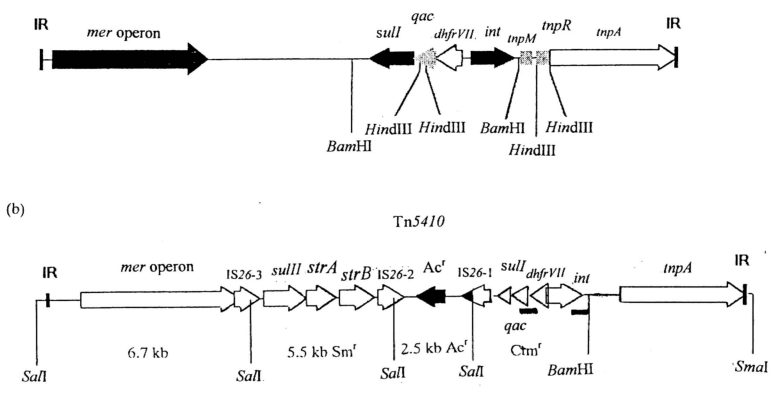

Figure 57 : Comparison between Tn*5086* and the proposed structure of *Tn5410*

(a) Transposon Tn*5086*

(b) A proposed structure of Tn*5410*. Genes that were sequenced in this study are indicated by solid arrows. Segments that were derived from pCL43 (from this study) and sequenced by Lye (1997) are indicated by solid bars.

and the Cm^r transposon.

4.5 Conclusion

Although there are many reports on the molecular characterization of transposons from *Salmonella* species, relatively little work has been reported on transposons from *S. typhi*. This study is the first to report the nucleotide sequence of an ampicillin resistance gene from a multiple antibiotic resistance transposon from *S. typhi*.

The ampicillin resistance gene of the transposon Tn*5410* studied appears to be part of a truncated Tn*2*-like element flanked by an IS*26*-like element and an RSF1010-like segment. Besides these characterized segments, circumstantial evidences suggest that Tn*5410* is a Tn*5086*-like element with additional DNA consisting of two other copies of IS*26*-like elements and a *sulIIstrAstrB* gene cluster like that of RSF1010. This transposon may be inserted in a Cm^r transposon on the R plasmid pST8 of *S. typhi* S8.

The history of emergence and spread of antibiotic resistance in bacteria shows that there are little we can do to arrest the development of resistance to currently used antibiotics and their successors. Transposons are the focal points for much of this genetic movement among microbes. This should force us to concentrate on the sensible use of antibiotics. Intelligent use of antibiotics can continue long after the emergence of resistance, provided vigilance is maintained with respect to monitoring the incidence of resistance. Perhaps the development of rapid PCR-based screening methods (Mabilat and Courvalin, 1990; Levesque *et al.*, 1995; Hannecart-Pokorni *et al.*, 1997) may allow us to monitor the transfer of and to characterize transposons an integrons.