CHAPTER FOUR DISCUSSION AND CONCLUSION

4.1 Detection of Ac’Ctm’Sm’ transposon and Cm’ 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’Ctm’Sm’ and the other at least Cm’ and probably Tc’ 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’ transposon was distinctly lower than that of the Ac’Ctm’Sm’ transposon. These results suggested that there were two different transposons on the R plasmids of *S. typhi*. These two transposons (i.e., the Ac’Ctm’Sm’ transposon and the Cm’ 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’Ctm’Sm’ transposon and the Cm’ transposon may be physically linked on the R plasmid of *S. typhi*. The results of all transposition experiments indicated that the Cm’ transposon most likely also carried another copy of the Ac’Ctm’Sm’ transposon. This is because the Ac’Ctm’Sm’ transposon was able to transpose independent of the Cm’ transposon but not vice versa; Cm’ transconjugants were usually generated when the transconjugants were selected for Ac’ or Ctm’ but Ac’Ctm’ transconjugants were usually generated when
transconjugants were selected for Cm'.

When Cm' transposed from the *S. typhi* plasmid to pUB307, it probably carried the Ac'Ctm'Sm' transposon along. Therefore, almost all the transconjugants obtained were Ac' and Ctm' when they were selected for Cm'. However, the Ac'Ctm'Sm' transposon, carried by the Cm' transposon on the *S. typhi* plasmid, could transpose to pUB307 independently and at a higher frequency than the Cm' transposon. Consequently, most transconjugants were Ac'Cm'Ctm'Km'Sm'Tc' when they were selected for Ac' or Ctm'.

Recombinant plasmids from Ac'Cm'Ctm'Km'Sm'Tc' transconjugants generated mostly Ac'Cm'Ctm'Km'Sm'Tc' transformants, indicating that these Cm' transconjugants harboured recombinant plasmids that carried the Ac'Ctm'Sm' transposon but not the Cm' transposon.

The Cm' 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' transposon to transpose to the chromosome could be due to the presence of many insertion sites on the chromosome. The transposon Tn7 is known to have such a site which is designated as att Tn7 (Lichenstein and Brenner, 1982). The Cm' transposon, carrying another copy of the Ac'Ctm'Sm' 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'Ctm'Sm' transposon carried by the Cm' transposon that had transposed onto the chromosome, might transpose back to pUB307, independent of the Cm' 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 \( \text{Ac}^\prime \text{Cm}^\prime \text{Ctm}^\prime \text{Km}^\prime \text{Sm}^\prime \text{Tc}^\prime \) were \( \text{Ac}^\prime \text{Cm}^\prime \text{Ctm}^\prime \text{Km}^\prime \text{Sm}^\prime \text{Tc}^\prime \). However, the \( \text{Ac}^\prime \text{Ctm}^\prime \text{Sm}^\prime \) transposon occasionally transposed via a ‘cut and paste’ mode, resulting in a loss of the \( \text{Ac}^\prime \text{Ctm}^\prime \text{Sm}^\prime \) phenotype from the \( \text{Cm}^\prime \) transposon on the chromosome. Consequently, an \( \text{Ac}^\prime \text{Cm}^\prime \text{Ctm}^\prime \text{Km}^\prime \text{Sm}^\prime \text{Tc}^\prime \) transconjugant was obtained (Table 9). The use of both the replicative and ‘cut and paste’ modes of transposition have been observed in Tn3-like transposons (Sherratt, 1989) and Tn7 (May and Craig, 1996).

The transposition frequencies of the two types of transposons, particularly the \( \text{Cm}^\prime \) transposon, were reduced when \( \text{E.coli} \) UB5201 harbouring the \( \text{S.typhi} \) plasmid and pUB307 was kept on Dorset egg slant for more than two weeks. The prolonged incubation could have allowed the \( \text{Cm}^\prime \) transposon on the \( \text{S.typhi} \) 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 \( \text{Cm}^\prime \) transposon.

4.2 Cloning of antibiotic resistance genes of pCL8

Previously, Kadambeswaran (1993) had detected an \( \text{Ac}^\prime \text{Ctm}^\prime \text{Sm}^\prime \) transposon from \( \text{S.typhi} \) S8. This transposon was subsequently designated as Tn5410 (Professor Koh C.L., pers. comm.). In this study, an \( \text{Ac}^\prime \text{Ctm}^\prime \text{Sm}^\prime \) transposon with similar transposition characteristics was also isolated from \( \text{S.typhi} \) S8. Therefore, it was assumed to be identical to Tn5410.

Based on the restriction mapping in Section 3.4.1, a copy of Tn5410 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'Ctm'Km'Sm'Tc' transconjugant. A 2.5 kb Ac' SalI fragment and a 5.5 kb Sm' SalI fragment were separately cloned into the plasmid pKan. Plasmid pKan (Micklos and Freyer, 1990) was used because it contains a unique SalI site and other plasmid vectors that do not carry Ac', Ctm', or Sm' were not available to me. The SalI-digested fragments of pCL8 were used for cloning because digestion of pUB307 with SalI generated only two large fragments. Furthermore, one of these SalI sites flanks Tn5410 in pCL8.

Among the five Ctm' 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 BamHI-site proximal to the Km' gene of the pKan vector was intact even after ligation with Sau3A1-digested fragments of pCLC600. In the process of constructing pKan from pUC19, Micklos and Freyer (1990) removed the restriction sites between BamHI and HindIII from the multiple cloning sites of pUC19 but the restriction sites between EcoRI and BamHI sites in the multiple cloning sites were retained. Therefore, the 4.3 kb insert of pCLC2 could be excised with an EcoRI-BamHI double digestion and subcloned easily into a better vector such as pUC19.

Among all the antibiotic resistance fragments cloned, the 2.5 kb Ac' SalI 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' SalI-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' SalI-insert was present in pST8. The labelled 2.5 kb Ac' probe also hybridized to the 1.6 kb fragment of the 1 kb ladder and the 4.2 kb SalI-digested pKan. The 1.6 kb fragment of 1 kb ladder is a Hpal-fragment of pBR322 and it contains the Ac' 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' gene derived from pUC19 (Micklos and Freyer, 1990).

The faint signal detected on the 5.5 kb Sm' SalI-fragment of pCL8 indicates that this fragment may have some similarity to the 2.5 kb Ac' probe. However, no signal was detected at the 5.5 kb region of SalI-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 Tn5410

4.4.1 The Tn2-like segment

Sequence similarity between the Tn2-like segment and Tn2 occurs between a stretch of 1444 nucleotides extending from the middle of tramR resolvase gene to the right inverted repeat (IR) of Tn2. The Ac' gene in this segment encodes the TEM-1 β-lactamase. This β-lactamase is identical to that found in Tn3, despite three silent nucleotide substitutions (Chen and Clowes, 1987).

Sequence variations among Tn1, Tn2, and Tn3 have been compiled by Chen and Clowes (1987) and Goussard and Courvalin (1991), and the nucleotide positions of Tn1
and Tn2 are assigned according to the nucleotide positions of Tn3. By comparison, the Ac<sup>f</sup> gene in Tnl differs from that of Tn3 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 Tn3 to Lys in Tnl. This difference accounts for the isoelectric focusing difference observed between the two β-lactamases from Tnl and Tn3 (Matthew et al., 1979). As a consequence, the β-lactamase from Tnl is designated the TEM-2-type (Matthew et al., 1979). The low divergence of 0.55 for the Ac<sup>f</sup> gene of Tnl, Tn2 and Tn3 suggests that this segment only diverged relatively recently (Chen and Clowes, 1987).

There is a single difference in the non-coding region of Tn2-like segment sequenced at position 754, i.e., a nucleotide G, instead of an A at position 3916 in Tn3 (Heffron et al., 1979) (Fig. 50a). This mutation is located near the promoter for β-lactamase transcription. Tn2 has only one β-lactamase transcription initiation site, which is identical to the P3 promoter of Tn3 (Brosius et al., 1982; Chen and Clowes, 1987). In Tnl, base substitution of T at position 3773 in place of a C in Tn3 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 Tn2 and Tn3 differ by a total of 22 bases, and 19 of them are clustered in the mpR gene segment, whereas Tnl and Tn3 differ by only three bases (Chen and Clowes, 1987). Based on the higher nucleotide divergence between the mpR genes of Tn2 and Tn3 (or Tnl) compared to that of the Ac<sup>f</sup> genes and the noncoding sequences in these transposons, Chen and Clowes (1987) proposed that the mpR
segment of Tn2 was derived from an earlier progenitor than the similar segments of Tn3 and Tn1. In this study, only one silent mutation was observed in each of the non-coding and \textit{mpR} regions sequenced, when compared to similar regions of Tn3. The nucleotide differences between the \textit{mpR} gene of Tn2 and Tn3 only occur at the third coding base of the codons. Therefore, no amino acid sequence changes resulted.

Truncated Tn3-like elements encoding Ac\textsuperscript{c} have been reported on numerous plasmids. Fayet \textit{et al.} (1982) found a plasmid that contains a 1.6 kb segment homologous to the Ac\textsuperscript{c} gene region of Tn2301, a Tn3-like transposon. This 1.6 kb DNA segment is not transposable, but when linked to the \textit{mpA} part of Tn2301 by ligation, it becomes a functional transposon. Plasmid pFM739, an RSF1010-like R plasmid from \textit{Neisseria sicca}, also contains a truncated Tn3-like element that is transposition defective (Rotger \textit{et al.}, 1986). The plasmid pBP749 was reported to carry a 1.18 kb segment that is homologous to Tn3 (Gerlach and Wiedemann, 1985). A segment that is 100% homologous to the Ac\textsuperscript{c},\textit{mpR} and part of \textit{mpA} of Tn1 was found in the multiple antibiotic resistance region of plasmid pCFF04 (Mabilat \textit{et al.}, 1992).

One ended transposition had been reported for Tn3 family transposon (Arthur \textit{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 Tn3 is about 1000 times less efficient than regular transposition (Ehrlich, 1989). Furthermore, the Tn2-like segment did not contain the sequences that encode the \textit{mpA} transposase. Therefore, it is unlikely that the integration of the Tn2-like segment into the progenitor of Tn5410 was mediated by a one-ended
transposition process. It is also unlikely that this Tn2-like segment was inserted into the progenitor of Tn5410 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 IS26-like element flanking the truncated end of the Tn2-like segment suggests that the Tn2-like element could have transposed into the progenitor of Tn5410 as an intact element and followed by deletion of the left segment of the Tn2-like element including the 38 bp IR sequence, _tmpA_ gene and part of the _tmpR_ gene that flanks the IS26-like segment sequenced. This hypothesis is supported by the presence of short sequence homology at the junction between the IR sequence of the IS26-like segment (adjacent to the Tn2-like segment) and Tn3 (Fig. 53). Wrighton and Strike (1987) also found such homology between IR ends of IS176 and the _tmpR_ gene of Tn1701 (Grindley and Nakada, 1981; Lambert _et al._, 1987; Cannon and Strike, 1992). IS176 is identical to IS26 (Barg _et al._, 1995) and Tn1701 to Tn2 (Yamada _et al._, 1979). Tn1701 was later designated as Tn4353 by Cannon and Strike (1992).

Although the junction between the IS26-like segment and the Tn2-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 IS26-like element could have inserted within the _tmpR_ gene and followed by a unidirectional deletion facilitated by sequence homology between the end of the IS26-like element and the putative deletion endpoint. This implies that the IS26-based transposase may have erroneously recognized the Tn2 sequences as a terminus of the IS26-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 IS26-like segment

The IS26 element has been reported under several pseudonyms, such as IS6 (Berg et al., 1975), IS15-delta (Trieu-Cout and Courvalin, 1984), IS46 (Brown et al., 1984), IS140 (Brau and Pieperberg, 1983), IS160 (Nies et al., 1985), and IS176 (Wrighton and Strike, 1987). This element is 820 bp long and carries 14 bp perfect terminal inverted repeats.

Insertion sequence IS15, formerly designated as IS1522 (Labigne-Roussel and Courvalin, 1983), is a close relative of IS26. It is formed by transposition of IS15-delta onto itself. The two IS elements that constitute IS15 have been designated as IS15-deltal and IS15-deltaII, which differ from IS26 at only 3 and 1 point substitutions, respectively, and are considered to be genetic variants of IS15-delta (or IS26) (Table 12). Homologous recombination between IS15-deltal and IS15-deltaII would lead to IS26 (Trieu-Cout and Caurvalin, 1984). Two other variants of IS26, designated as IS15-deltaIII (Ouellette et al., 1987) and IS15-deltaIV (Nucken et al., 1990), differ from IS26 at only one point substitution each (Table 12). In this study, it is not known if the IS26-like element discovered is identical to IS26 or its variants because the segment cloned and sequenced is common to IS26 and its variants.

The promoter of the IS26 transposase gene is located within an IR of IS26 and is found in the IS26-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 IS26 and its variants

<table>
<thead>
<tr>
<th>Nucleotide position</th>
<th>IS26</th>
<th>IS15 delta I</th>
<th>IS15 delta II</th>
<th>IS15 delta III</th>
<th>IS15 delta IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>459</td>
<td>G</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>563</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>A</td>
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<tr>
<td>613</td>
<td>G</td>
<td>A</td>
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<td>G</td>
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</tr>
<tr>
<td>614</td>
<td>G</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>764</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>T</td>
</tr>
</tbody>
</table>

Note: The nucleotide position is based on the sequence of IS26R (Mollet et al., 1983)
1997). This transposase 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).

IS26-like elements contain unique PstI and SacI sites at position 159 and 252, respectively (Mollet et al., 1983). These two sites were found on the IS26-like segment sequenced. For ease of explanation, henceforth the IS26-like segment sequenced, which extends from the SacI site to the IR end, will be designated as the left segment IS26R (Fig. 55). The 'R' here refers to the IS26 that is located on the right end of the composite transposon Tn2680 (Mollet et al., 1983).

Besides the Km' transposon Tn2680, which is identical to Tn6 (Berg et al., 1975), other IS26-based elements are also found in other Km' composite transposons, such as Tn1525 (Trieu-Cout and Courvalin, 1984) and Tn4352 (Wrighton and Strike, 1987). IS26-based composite transposons carrying other known antibiotic resistance genes include the Ac'Gen' Tn2922 (Martin et al., 1987) and the Tp' Tn5091 (Sundstrom et al., 1995) and its unnamed Tp' relative (Barg et al., 1995), are flanked by IS76, i.e., IS26. IS26 has relatives that are similar but not identical. One of these relatives is the IS6100 on a Su' transposon of Mycobacterium fortuitum (Martin et al., 1990).

Interestingly, in all IS26-based composite transposons reported above, the IS26-like elements are arranged in direct repeats. When three copies of IS26 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 IS26 are thought to be essential for the transposition
**Figure 55:** The structure of IS26

**Figure 56:** The *sull strA strB* gene cluster of RSF1010
of IS26-based composite transposon. IS26 elements require a cointegrate formation step that involves one copy of the IS26 and a resolution step that involves recA dependent homologous recombination between the directly repeated copies (Galas and Chandler, 1989). Therefore, IS26-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' Sall-fragment that is adjacent to the 2.5 kb Ac' Sall-fragment in pCL8. The location of the RSF1010-like segment, which is between the Tn2-like segment and the adjacent 5.5 kb Sm' Sall fragment, and its orientation suggest that the Sm' gene or genes in the 5.5 kb Sm' Sall fragment are similar to the Su' (sulII) Sm' (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' fragment to the Sall site and from the Sall site to the 5.5 kb Sm' fragment is sufficient to accommodate a copy of IS26-like element inserted in either direct or inverse orientation with respect to the copy of IS26-like element on the other terminus of the 2.5 kb Ac' fragment.

Based on the above assumption that the Sm' determinants in Tn5410 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' Sall-EcoRI fragment from the 5.5 kb Sm' insert of pCLS55. As expected, the Sm' 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 *sulI strA strB* is driven by the P₅ and P₆ promoters located at the 5' end of the *sulII* gene.

Miss R.P. Pillai also found that the characteristic *PstI* site of IS26R (Fig. 55) was absent near the *Sall*-terminus of the 2.6 kb Sm′ *Sall-EcoRI* fragment that she subcloned. We may deduce that if a copy of IS26-like element is inserted in the junction between the 2.5 kb Ac′ and 5.5 kb Sm′ fragments, then this copy should be inserted in the inverse orientation to the IS26-like element (designated as IS26-1) on the other terminus of the 2.5 kb Ac′ fragment. Although nucleotide sequences beyond the RSF1010-like segment have not been determined, the putative second copy of the IS26-like element (designated as IS26-2) is assumed to be inserted in the reverse orientation to the first, in the following discussion.

Based on this assumption, the putative IS26-like segment on the 5.5 kb Sm′ fragment adjacent to the 2.5 kb Ac′ fragment may be a left segment of an IS26R-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′ fragment probe contained the right segment of IS26R. Therefore, a third copy of IS26-like element (designated as IS26-3) may be present on the other terminus of the 5.5 kb Sm′ fragment and is in direct orientation to the second copy.

4.4.4 The structure of Tn5410

Recently, Lye (1997) has subcloned a 2.2 kb Tp′ *HindIII-BamHI* fragment from the 4.3 kb Ctm′ insert of pCLC2. Partial sequencing of this fragment from both termini showed that nucleotide sequences at the *BamHI* terminus are almost identical to the
integrase (int) gene of the integron of Tn5086 and Tn21 (Sundstrom et al., 1993) and the nucleotide sequences at the HindIII terminus are almost identical to part of the Tp' (dhfrVIII) and qacEDA gene cassettes of Tn5086. The int genes in Tn5086 and Tn21 are almost identical except for a group of three consecutive G's close to the start of the int gene in Tn21 which is missing in Tn5086 (Mercier et al., 1990; Sundstrom et al., 1993). The qacEDA gene cassette is part of the conserved region of sulII associated integron (Stokes and Hall, 1989; Paulsen et al., 1993).

These findings suggest that Tn5410 may be closely related to the Tn21-like transposon, Tn5086 (Fig. 57a). Transposon Tn5086, like most Tn21-like transposons, confers resistance to HgCl₂. However, when S. typhi S8 was tested on an LB plate containing HgCl₂ (50 μg/ml), no growth was observed. Therefore, it is possible that the putative third copy of IS26-like element (designated as IS26-3) may have inserted into the mercury resistance operon of a Tn5086-like progenitor of Tn5410. A genetic organization of Tn5410 is proposed here (Fig. 57b).

The 6.7 kb SalI fragment of pCL8, adjacent to the 5.5 kb Sm' fragment, is yet to be characterized. It may contain the putative mercury resistance operon of the Tn5086-like element.

The Cm' transposon has not been isolated yet and the presence of Tc' on this transposon has not been confirmed. A multiple antibiotic resistance transposon comprising a cluster of resistance genes, including Cm' and Tc', interposed by multiple copies of IS26-like elements, i.e., (IS26 or IS15)-Cm'-ISI-Sul'-Sp'-IS26-Tc'-IS26-Ac'- (IS26 or IS15), 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 Tn5410
Figure 57: Comparison between Tn5086 and the proposed structure of Tn5410

(a) Transposon Tn5086

(b) A proposed structure of Tn5410. 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\textit{5410} studied appears to be part of a truncated Tn2-like element flanked by an IS26-like element and an RSF1010-like segment. Besides these characterized segments, circumstantial evidences suggest that Tn\textit{5410} is a Tn5086-like element with additional DNA consisting of two other copies of IS26-like elements and a *sulII strA strB* 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.