Molecular divergence of the serotype-specific plasmid (pSLT) among strains of *Salmonella typhimurium* of human and veterinary origin and comparison of pSLT with the serotype specific plasmids of *S. enteritidis* and *S. dublin*

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Summary. Molecular variants of the serotype-specific plasmid (SSP) of *Salmonella typhimurium* (pSLT) were recognised in clinical and veterinary isolates by restriction enzyme fingerprinting. Three had undergone minor DNA rearrangements, whereas two had acquired resistance determinants to a wide range of antimicrobial agents including gentamicin, trimethoprim, tetracycline, streptomycin, ampicillin (Ap) and kanamycin (Km). One of the latter was the result of co-integrate formation with an IncX, conjugative R-plasmid that specified ApKm resistance. The co-integrate plasmid (pOG669) was incompatible with, and displaced, pSLT and its molecular variants. The restriction fingerprints of SSPs of *S. enteritidis* and *S. dublin* were compared with pSLT. All were related at the 35% level on the basis of a Dice coefficient of similarity. The SSPs of *S. enteritidis* and *S. dublin* were incompatible with the co-integrate plasmid pOG669. Whereas in *S. enteritidis* this resulted from incompatibility with the pSLT component (the SSP was compatible with the IncX component), the converse was found with *S. dublin*.

Introduction

Several independent studies have shown that certain serotypes of *Salmonella* harbour serotype-specific plasmids (SSPs) (Helmuth *et al.*, 1985). The most notable serotypes were *S. typhimurium* (Jones *et al.*, 1982), *S. enteritidis* (Nakamura *et al.*, 1985) and *S. dublin* (Terakado *et al.*, 1983). Popoff *et al.* (1984) demonstrated a high level of molecular relatedness between the SSPs of *S. typhimurium* and *S. dublin* by hybridisation and Baird *et al.* (1985) showed extensive homology between the regions of these plasmids associated with virulence.

The SSPs of salmonellae have not been assigned to an established incompatibility group (Inc). The plasmids MP10 (probably synonymous with pSLT) and A* were studied by Anderson and Smith (1972) who demonstrated their compatibility with IncF, F₁ and H₁. They were not compatible with F₁me plasmids. Willshaw *et al.* (1978), on the basis of DNA reassociation studies, concluded that their

incompatibility did not reflect a close phylogenetic relationship.

The SSP of *S. typhimurium* was designated pSLT by Jones *et al.* (1982), who used *S. typhimurium* LT2 as their reference strain. In an earlier report (Brown *et al.*, 1986) we showed that this plasmid was readily recognised by restriction enzyme fingerprinting with *Pst*I and *Sma*I and was present among most, but not all, of the phage types of *S. typhimurium* examined. Detailed comparison of the fingerprints from clinical isolates with that of pSLT was restricted to those isolates that harboured a single plasmid and thus could be interpreted unequivocally. In each of 15 cases the plasmid was identical with pSLT on the basis of *Pst*I and *Sma*I fingerprints and we concluded that the SSP of *S. typhimurium* was remarkably conserved.

In this study we have extended previous investigations, examined a wider range of bacterial strains, and further defined the interpretation of fingerprint data to demonstrate molecular divergence of the SSP of *S. typhimurium*. The restriction fingerprints

of SSPs from *S. typhimurium*, *S. enteritidis* and *S. dublin* have also been compared with each other together with certain of their incompatibility properties.

Materials and methods

Bacterial strains and plasmids

Clinical isolates of *S. typhimurium*, *S. enteritidis* and *S. dublin* were from the departmental collection which came from various laboratories throughout Scotland including the Microbiology Department, Royal Hospital for Sick Children, Glasgow (RHSC); Veterinary Investigation Centre, West of Scotland Agricultural College, Auchincruive; and the Scottish Salmonella Reference Laboratory (SSRL), Stobhill Hospital, Glasgow. SSRL had confirmed the serotype and phage type of the *S. typhimurium* isolates.

S. typhimurium LT2 (Zinder and Lederberg, 1952) ATCC 235564 was used as a source of the plasmid pSLT. In view of its early isolation, ready availability and widespread usage, this plasmid was chosen to provide reference restriction enzyme fingerprints. However, it was also compared with the indigenous plasmid (designated pOG660) of *S. typhimurium* NCTC 73 which was isolated in 1917 and antedates LT2. *E. coli* K12 J53-2 and J62-1 were used as recipient strains for plasmid transfer from *S. typhimurium* (Platt *et al.*, 1984). *E. coli* K12 J62::Tn7 was kindly provided by Dr Hilary Richards as a chromosomal source of the transposon Tn7. The IncX plasmid, Tp231, was obtained from the National Collection of Type Cultures and used to confirm the Inc

group of pOG670 and the dual incompatibility properties of pOG669. Bacterial strains are shown in table I.

Construction of plasmid pOG664

Plasmid pOG670 was transferred from *S. typhimurium* GRI31486 to a *lac*⁻ strain of *E. coli* K12 that encoded a chromosomal copy of Tn7 (J62::Tn7). Further conjugation to a rifampicin resistant *lac*⁺ *E. coli* (J53-2) with trimethoprim and rifampicin selection allowed the isolation of the plasmid pOG670::Tn7 which was designated pOG664 and used to test the dual incompatibility of pOG669.

Construction of co-integrate plasmid pOG665

Plasmid pOG670 was transferred from *S. typhimurium* GRI31486 to an *E. coli* K12 auxotroph, and then by further conjugation to *S. typhimurium* LT2, by use of minimal medium and ampicillin selection; this strain was designated LT2-4. *S. typhimurium* LT2-4 was successively subcultured six times on CLED agar at 40°C with ampicillin (Ap) and kanamycin (Km) selection. Single colonies were examined for the presence of a co-integrate plasmid (pOG665) by agarose gel electrophoresis, as described below.

Plasmid profiles and restriction fingerprints

Plasmid DNA was examined in crude lysates by a modification of the method of Platt and Sommerville (1981). The equivalent of 12–20 colonies of an overnight

Table I. Bacterial strains and plasmids

Species and designation	Phage type (DT)	Plasmid profile (mol. wt in kb)	Plasmid designation	Source or reference
<i>S. typhimurium</i>				
NCTC 73	9	87	pOG660†	Isolated in 1917
LT2	4	87	pSLT	Brown, Munro and Platt, 1986
LT2—4	NT	87, 52	pSLT, pOG670	This paper
LT2—5	NT	139	pOG665	
GRI 37286	12	87	pOG666	Human strains from RHSC isolated between 1977 and 1984
GRI 37386	12	87	pOG667	
GRI 37486	RDNC	87	pOG668	
GRI 37586	204C	87*, 52, 4.3	pOG666*	
GRI 37686	204C	87*, 52, 4.3	pOG666*	
GRI 30986	204C	174, 139*, 87	pOG669	Bovine strains isolated in 1985
GRI 31486	204C	174, 87, 52*, 7.2	pOG670*	
GRI 31986	204C	101*, 4.3	pOG672*	
<i>S. enteritidis</i>				
GRI 16485	NT	52	pOG674	Human strains isolated in 1985
<i>S. dublin</i>				
GRI 34285	NT	72	pOG675	

NT = not tested.

*Indicates the approximate mol. wt of the designated plasmid in strains that harboured more than one plasmid.

† Indistinguishable from pSLT on the basis of *Pst*I, *Sma*I and *Ava*II fingerprints

culture on Nutrient Agar (Oxoid, CM3) was suspended in 300 µl of electrophoresis buffer (EB) (89 mM Tris, 89 mM boric acid, 1.25 mM EDTA, pH 8.2) in 1.5-ml Eppendorf tubes; 200 µl of sodium dodecyl sulphate (SDS) 10% w/v in EB were added and the tubes were mixed gently by inversion. After being heated at 50°C for 5 min, the crude lysates were centrifuged for 15 min (9980 g) in a micro-centrifuge. The supernatant fraction (100 µl) was loaded on to a vertical agarose gel (0.7%, Sigma, type II) together with 5 µl of tracking dye (sucrose 25%, 8 mM sodium acetate, 3.5 mM, SDS, 0.7 mM bromophenol blue). Electrophoresis was carried out for 1 h at 100 V followed by 4 h at 200 V (constant voltage). Gels were stained with ethidium bromide 6 µg/ml for 15 min, viewed with a UV transilluminator (365 nm) and photographed on type 665 film (Polaroid). The mol. wts of plasmids were determined by reference to plasmids of known mol. wt; Rts 1 (174 kb), RA-1 (123 kb), R1 (90 kb), R702 (67 kb) and RP4 (52 kb). Supercoiled ladder (Gibco-BRL, Paisley) was used for the mol.-wt estimation of small plasmids (<15 kb). Mol.-wt values incorporated into plasmid profiles were determined on a minimum of two occasions.

Restriction fingerprinting was performed as previously described in detail (Platt *et al.*, 1986). Plasmid DNA from clinical isolates and transconjugants was extracted and purified by a modification of the method of Birnboim and Doly (1979). Restriction enzymes were obtained from Gibco-BRL except *Bsp*1286 (CP Laboratories, Bishops Cleeve, Herts) and used according to the manufacturer's instructions. Restriction fingerprints were compared by using a coefficient of similarity (Dice, 1945) calculated from the formula $S_D (\%) = [2m/(a+b)] \times 100$, where 'm' was the number of restriction fragments common to two plasmids (A and B), and 'a' and 'b' were the total numbers of fragments generated

from each plasmid after digestion by the same restriction enzyme.

Interpretation of restriction fingerprints

The following general rules were applied to the interpretation of plasmid fingerprints:

- (1) To establish that an observed fingerprint represented a variant of pSLT demanded that its recognition was initially in a strain of *S. typhimurium* in which it was the sole plasmid or was similarly present in an *E. coli* transconjugant. In the latter situation each of the fragments detected must have been present in the original isolate of *S. typhimurium*.
- (2) If the difference between the observed variant fingerprint and pSLT was solely due to an additional fragment or fragments the same result must have been obtained when the plasmid was digested with twice the standard amount of restriction enzyme to exclude the presence of the products of partial digestion.
- (3) Subsequent presumptive recognition of variants in clinical or veterinary isolates that contained additional plasmids was accepted if the additional plasmid was substantially different in copy number (Platt and Taggart, 1987) or if the variant had lost at least one fragment.

Incompatibility properties determined on the basis of interaction with plasmid pOG669

Dual incompatibility properties of the naturally occurring co-integrate plasmid, pOG669 (Platt, 1987), were confirmed on the basis of interactions with Tp231 (IncX), pOG664 and pOG672, a resistant derivative of pSLT (table II). Plasmid pOG669 was used to investigate the

Table II. Summary of characteristics of the plasmids studied

Plasmid	Transferability to <i>E. coli</i> K12	Resistance determinants	Incompatibility with		Origin
			pOG669	pOG670	
pOG664	+	Ap Km Tp	+	NT	pOG670::Tn7 pOG670/p SLT co-integrate
pOG665	+	Ap Km	NT	NT	
pOG666	—	—	+	—	naturally occurring variants of pSLT
pOG667	—	—	+	—	
pOG668	—	—	+	—	
pOG669	+	Ap Km	NT	NT	
pOG670	+	Ap Km	NT	NT	IncX R-plasmid
pOG672	+	Tc Sm Gm Tob Tp	+	—	naturally occurring variant of pSLT
pOG673	+	Tc Sm Gm Tob Tp	+	—	conjugational rearrangement of pOG672
pOG674	—	—	+	—	SSP <i>S. enteritidis</i>
pOG675	—	—	+	+	SSP <i>S. dublin</i>
pSLT	—	—	+	—	SSP <i>S. typhimurium</i> LT2
Tp231	+	Ap Tc Cm	+	+	IncX reference plasmid

Ap, ampicillin; Km, kanamycin; Tp, trimethoprim; Tc, tetracycline; Sm, streptomycin; Gm, gentamicin; Tob, tobramycin. NT = not tested.

incompatibility properties of plasmids related to pSLT and the SSPs of *S. enteritidis* and *S. dublin* as follows: pOG669 was introduced into a strain of each serotype by conjugation, and selected for by Ap or Km resistance; the presence of both plasmids in transconjugants was confirmed by agarose gel electrophoresis. After unselected growth, segregation was detected by disk-diffusion sensitivity testing. ApKm-resistant clones remained stable with respect to these determinants and harboured only the 139-kb plasmid whereas a *Sma*I fingerprint of ApKm sensitive clones confirmed the presence of only the resident plasmid.

Plasmid pOG670 (the IncX, ApKm conjugative R-plasmid component of pOG669) was investigated in parallel to establish whether any incompatibility observed was a consequence of the pSLT component of the co-integrate.

Mobilisation of plasmid pOG672

The RP1 derivative plasmid pMR5 is temperature-sensitive for replication (Robinson *et al.*, 1980). It was transferred into *E. coli* K12 J53-2, which already contained plasmid pOG672, by conjugation and was selected for with rifampicin and kanamycin. This strain was used to transfer pOG672 into *S. dublin* GRI 34285 which already contained plasmid pOG675, minimal medium being used to counterselect against the plasmid donor and trimethoprim resistance to select for incoming pOG672. Transconjugants were grown at 37°C to effect the elimination of pMR5, which was confirmed by loss of ApKm resistance. Agarose gel electrophoresis was used to confirm that pOG675 and pOG672 were present in *S. dublin*.

Results

Occurrence of serotype-specific plasmids

Seven isolates of *S. typhimurium* from human or veterinary sources harboured plasmids which gave *Pst*I or *Sma*I fingerprints, or both, related to but different from that obtained with the serotype-specific plasmid (pSLT) of *S. typhimurium* LT2. A plasmid indistinguishable from pSLT was demonstrated in *S. typhimurium* NCTC 73. The characteristics of the isolates are shown in table I and the plasmids in table II. Plasmids pOG666, 667 and 668 were all detected in human isolates which harboured a single plasmid. *Pst*I and *Sma*I fingerprints are shown in fig. 1, together with pSLT; pOG666 differed from pSLT only in the *Sma*I fingerprint, pOG668 only in the *Pst*I fingerprint and pOG667 in both *Pst*I and *Sma*I fingerprints. Whereas pOG666 was first recognised in a strain of *S. typhimurium* that belonged to phage type (DT)12, it was also presumptively identified in two isolates of DT 204c which harboured additional plasmids.

a b c d e f g h i j k l m n o p q

Fig. 1. Tracks a-d and e-h: *Pst*I and *Sma*I digests, respectively, of pSLT, pOG668, pOG667 and pOG666. Tracks i, j, o: *Pst*I, *Sma*I and *Ava*II digests of bacteriophage λ . Tracks k, l, m, n, p, q: pOG672 and pOG673 digested with *Pst*I, *Sma*I and *Ava*II respectively. Fragments shown range from 1 kb to 19 kb.

Plasmid pOG669 was first recognised during the investigation of an outbreak of salmonellosis among cattle. *S. typhimurium* GRI 30986 harboured three plasmids (Plasmid profiles 120, 96, 60). *Pst*I and *Sma*I fingerprints of the original isolate indicated that pSLT was present. After R-transfer the analysis of transconjugants indicated that the 87-kb plasmid specified resistance to streptomycin, gentamicin and tobramycin and bore no resemblance to pSLT in *Pst*I and *Sma*I fingerprints. However, fingerprints of the 139-kb plasmid that specified ampicillin and kanamycin resistance contained all the restriction fragments of pSLT together with several additional fragments. Furthermore, a 52-kb IncX plasmid (pOG670) that specified ApKm resistance had been demonstrated in other *S. typhimurium* isolates from the same outbreak, the restriction fragments of which corresponded to the additional fragments found in pOG669. These results strongly suggested that the formation of pOG669 was the result of co-integration between pSLT and pOG670. This was confirmed by the construction of pOG665 *in vitro*. The restriction fingerprints of pOG669 and pOG665 were very similar but not identical and indicated that the co-integration site was different in the natural co-integrate and that constructed *in vitro*.

Fig. 2 shows *Pst*I, *Sma*I and *Ava*II fingerprints of the co-integrate plasmid pOG669 together with

Fig. 2. Tracks a, k, l: bacteriophage λ digested with *Pst*I, *Ava*II and *Sma*I respectively. Tracks b, e, h show pOG670, tracks c, f, i show pOG669, and tracks d, g, j show pSLT digested with *Pst*I (b, c, d), *Sma*I (e, f, g) and *Ava*II (h, i, j).

those of pOG670 and SLT from which it was derived.

Plasmids pOG672 and pOG673

When conjugative R-transfer was performed from *S. typhimurium* GRI 31986 (table I), transconjugants were obtained in *E. coli* K12 from both tetracycline and streptomycin selection plates. Each harboured a single plasmid of about 101 kb, although a new plasmid (pOG673) was consistently smaller than pOG672. Each specified the same resistance determinants including gentamicin (table II) and although the restriction fingerprints were very similar, differences were detected. Fig. 1 shows *Pst*I, *Sma*I and *Ava*II fingerprints of both pOG672 and pOG673. With each enzyme, restriction fragments present in the fingerprints of pOG672 were absent from those of pOG673. Whereas all the restriction fragments of pOG672 in *E. coli* K12 matched fragments in a digest of *S. typhimurium* GRI 31986, pOG673 lacked fragments present in both. Thus, pOG672 was the 'wild type' plasmid and pOG673 a deletion derivative of it obtained during conjugation with *E. coli* K12.

The *Pst*I, *Sma*I and *Ava*II fingerprints of variant plasmids were compared quantitatively with that of pSLT by use of the Dice coefficient (S_D) of similarity. Three of the variants (pOG666, 667 and 668) have diverged minimally from pSLT and had S_D values >90%. Plasmids pOG672 and pOG673

produced lower S_D values (76% and 75% respectively) which reflect the increase in the mol. wt of these plasmids. The S_D values obtained when pOG669 was compared with pSLT were similar (mean 77% for the three enzymes), despite a larger increase in the mol. wt. Although pOG670 contributed significantly to the total mol. wt of the co-integrate, the S_D value was higher than might have been expected. This was because pOG670 contributed fewer restriction fragments, in proportion to its mol. wt, than did pSLT.

Incompatibility studies with plasmid pOG669

Plasmid pOG669 was incompatible with both Tp231, a reference IncX plasmid, and pOG672 a naturally occurring resistant derivative of pSLT. In each case selection for the incoming plasmid (Tp231 and pOG672) resulted in the isolation of transconjugants that harboured both plasmids. Subsequent growth in the absence of selection pressure for either plasmid resulted in bi-directional segregation; pOG669 was the more stable in both plasmid combinations. However, when pOG664 was introduced into a strain that harboured pOG669, no transconjugants harboured both plasmids; pOG669 was consistently displaced by pOG664.

When pOG669 was introduced into *S. typhimurium* LT2 or strains that harboured one of the variant plasmids described above, the plasmids were incompatible (table II). Similar results were obtained with *S. enteritidis* and *S. dublin*. However, to exclude the possibility that incompatibility was the result of interaction between the IncX component of the co-integrate and the resident plasmid, pOG670 was introduced separately into each strain. With the exception of *S. dublin*, pOG670 was compatible with each of the resident plasmids. The SSP of *S. dublin* (pOG675) therefore exhibited incompatibility with both pOG669 and pOG670. To distinguish between dual incompatibility and IncX properties alone, pOG672 was mobilised into *S. dublin* by pMR5 which was subsequently eliminated by growth at the non-permissive temperature; pOG675 was incompatible with pOG672 and indicated IncX incompatibility alone.

Relationships between the SSPs of *S. typhimurium*, *S. enteritidis* and *S. dublin* on the basis of quantitative comparison of restriction fingerprints

Visual inspection of the fingerprints obtained after digestion with *Pst*I and *Sma*I indicated little overall similarity. The fragmentation patterns were readily identified and distinguished. However

Table III. Molecular relatedness of variants of the *S. typhimurium* SSP to plasmid pSLT from *S. typhimurium* LT2

Restriction enzyme used to obtain fingerprint	Dice coefficient of similarity (%) after comparison of pSLT with molecular variants of the <i>S. typhimurium</i> SSP							
	pOG666	pOG667	pOG668	pOG669	pOG670*	pOG672	pOG673	
					S†		S†	
<i>Pst</i> I	100	91	95	73	5	0	75	96
<i>Sma</i> I	91	93	100	76	30	7	82	92
<i>Ava</i> II				82	47	9	71	95

* Although not a molecular variant of pSLT this plasmid was included on the basis that together with pSLT it formed the co-integrate plasmid pOG669

S† The similarity coefficient obtained by comparing the plasmids on the left and right of the boxes.

quantitative comparison by use of S_D values and by including two additional enzymes (*Ava*II and *Bsp*1286) showed that each of the plasmids was related at about the 35% level (table IV). These results indicate that the SSPs of *S. enteritidis*, *S. dublin* and *S. typhimurium* have all diverged to a similar extent and also that the relationship between the SSPs of *S. enteritidis* and *S. dublin* was no closer than each of them to *S. typhimurium*.

Discussion

Plasmid profile analysis has become established as an important tool in epidemiological investigations (Hawkey, 1987). Implicit in this application of plasmid detection is the diversification of plasmid profiles within and between outbreaks of infectious disease (Farrar, 1983). Recently, however, it has been recognised that some plasmids, notably those

associated with enhanced pathogenicity, are particularly stable and their demonstration confers negligible epidemiological specificity. These include the serotype-specific plasmids of salmonellae (Brown *et al.*, 1986; Platt, 1987), R-plasmids of *S. wein* (Casalino *et al.*, 1984) and several plasmids of *Shigella sonnei* (Prado *et al.*, 1987). The results presented here demonstrate that various different molecular rearrangements have taken place in the SSP of *S. typhimurium* and these may restore a degree of epidemiological specificity. Molecular variants, which may include point mutations, were readily recognised in *Pst*I or *Sma*I restriction enzyme fingerprints, or in both. This indicates that plasmid fingerprinting can resolve differences that remain undetected in plasmids profiles, and demonstrates that similarity of mol. wt is not a prerequisite of relatedness among SSPs of *S. typhimurium*. The recognition of molecular variants can be reconciled with our previous observation that *Pst*I and *Sma*I fingerprints were highly conserved in a representative collection of 96 strains of *S. typhimurium* isolated during 1985 on the basis that the variant plasmids were detected among salmonellae that were isolated from either paediatric infections collected over a 7-year period or veterinary isolates associated with an outbreak during 1985.

Although the molecular mechanisms that gave rise to the pSLT variants pOG666, 667 and 668 are unknown, pOG669 is clearly the result of co-integration between pSLT and pOG670 (Platt, 1987, and this report). The behaviour of the latter plasmid supports some but not all of the proposals of Bennett *et al.* (1986); although it exhibits dual incompatibility properties, in the absence of selection pressure they are not unidirectional.

There are several clinical implications of the co-

Table IV. Molecular relatedness of SSPs from *S. typhimurium*, *S. enteritidis* and *S. dublin* on the basis of restriction fingerprints

Serotypes from which SSPs were compared	Dice coefficient of similarity (%) for fingerprints obtained with				Mean coefficient
	<i>Pst</i> I	<i>Sma</i> I	<i>Ava</i> II	<i>Bsp</i> 1286	
<i>S. typhimurium</i> and <i>S. enteritidis</i>	21	38	46	39	36
<i>S. typhimurium</i> and <i>S. dublin</i>	22	41	32	34	32
<i>S. enteritidis</i> and <i>S. dublin</i>	25	50	39	46	37.5

integration event. Firstly, the co-integrate plasmid is freely autotransmissible *in vitro* to a wide range of *Salmonella* serotypes, including those that lack SSPs. Secondly, when pOG669 was transferred to strains of *S. typhimurium* that harboured pSLT, the latter plasmid was displaced by incompatibility. Preliminary studies (Brown *et al.*, unpublished data) have shown that strains that harboured either plasmid were equally pathogenic in mouse virulence tests, which suggests that co-integration has not diminished any virulence properties conferred by pSLT. Thirdly, the linkage of autotransmissibility, drug resistance and virulence properties may allow the dissemination of this plasmid among other organisms with an attendant increase in their pathogenic potential.

Restriction enzyme fingerprinting, in contrast to hybridisation, provides an indirect estimate of plasmid relatedness. Farrar (1983) has argued that if two plasmids generate identical restriction fragments, from two or more enzymes, they may be assumed to be identical plasmids; conversely, plasmids of the same size that produce entirely different fragmentation patterns are essentially unrelated plasmids; and, finally, plasmids of the same or different sizes may share homologous regions of DNA, which are detectable in restriction fingerprints. We have extended this argument to include a quantitative estimate of plasmid relatedness based on the Dice coefficient of similarity where the restriction enzymes are chosen to produce an optimal number of fragments (Platt *et al.*, 1986).

In this study, such estimates were based on a minimum of 50 fragments (pOG666, 667 and 668) and substantially more where three or four enzymes were used (tables III and IV). Although useful, this approach is not without certain limitations and the results should be interpreted with caution. For example, when pOG669 and pOG672 were com-

pared with pSLT the coefficients of similarity were 78 and 77% respectively (based on a mean of three enzymes) which suggested that each plasmid had diverged equally from pSLT. Although both plasmids contained additional DNA, pOG669 was about 36 kb larger than pOG672. Thus, when comparing plasmids, one of which contains a DNA insert, similarity coefficients are not only influenced by the size of the insert, but also by the number of restriction sites it possesses for the enzyme(s) used in their determination. Such problems do not occur when dealing with plasmids that differ only by deletions.

Popoff *et al.* (1984) concluded that the SSPs of salmonella, including *S. typhimurium*, *S. enteritidis* and *S. dublin*, represented a family of related plasmids on the basis of hybridisation studies and *Hind*III fingerprints. The data from *Pst*I, *Sma*I, *Ava*II and *Bsp*I286 fingerprints are consistent with this conclusion. The incompatibility studies lend further support with respect to *S. typhimurium* and *S. enteritidis* but the findings with *S. dublin* were unexpected and not easily reconciled with either hybridisation (Popoff *et al.*, 1984; Baird *et al.*, 1985) or fingerprinting results. The role of these plasmids in virulence would lead to the conservation of those regions associated with virulence. If the SSP of *S. dublin* had undergone co-integration with an IncX plasmid at some stage in its history (analogous to the natural formation of pOG669), the maintenance of selection for virulence need not have co-selected for conservation of a pSLT incompatibility determinant; its subsequent deletion, extending into other regions, would then explain the reduced size, incompatibility and low level of fingerprint similarity compared with pSLT. Loss of this incompatibility determinant was observed among F₁me plasmids (McConnell *et al.*, 1979) and would support this hypothesis.

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