Genotypic typing and phylogenetic analysis of *Salmonella paratyphi* B and *S. java* with IS200

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Salmonella paratyphi B and *Salmonella java* are biovars of common serotype 1,4,[5],12:b:1,2 which respectively cause human paratyphoid fever and gastroenteritis. In order to define genotypes and phylogenetic relationships in this group, we examined representative strains for restriction fragment length polymorphisms (RFLPs) in and around the 16S ribosomal RNA (*rrn*) genes, and the five to eleven insertion sites of the *Salmonella*-specific DNA insertion sequence IS200. One of four 16S *rrn* profiles was predominant, and was shared by the majority of strains, irrespective of their designation as *S. paratyphi* B or *S. java*. On the other hand, thirteen unique IS200 profiles were found and this technique was able to distinguish, for the first time, distinct genotypes for *S. paratyphi* B and *S. java*. One of the *S. paratyphi* B profiles, *Spj*-IP1.0, represented a globally-distributed clone. Greater diversity was detected within IS200 profiles of *S. java* than within those of *S. paratyphi* B. IS200 profiles described a phylogenetic complex in which strains of both biovars could be placed. They constituted reproducible molecular fingerprints, which could be compared in a band-matching database suitable for molecular epidemiological typing.

Introduction

Two groups of *Salmonella* within serogroup B share the same somatic antigen profile 1,4,[5],12 and flagellar antigens b:1,2. Strains of the first group, which are unable to ferment dextrorotatory tartrate (dTa⁻) and produce a slime wall, cause paratyphoid fever in man. Strains of the second group ferment dextrorotatory-tartrate (dTa⁺), lack a slime wall, and cause gastroenteritis in either man or animals. Kauffmann (1955, 1966) named the dTa⁻ human-adapted paratyphoid isolates as *S. paratyphi* B, and the dTa⁺ gastroenteritis isolates as *S. java*. Le Minor *et al.* (1982) subsequently proposed that the appropriate description of the latter group was *S. paratyphi* B biovar *java*.

Barker *et al.* (1988) employed phenotypic and rRNA typing to distinguish three groups among all the strains sharing this serotype. Selander *et al.* (1990a, b) employed

multilocus enzyme electrophoresis (MLEE) to distinguish 14 electrophoretic types (ETs) within the serovar, observing considerable genotypic diversity among ETs, and concluding that most dTa⁻ strains comprised a globally-distributed clone with polymorphic phenotypes. Neither of these approaches was able to define a distinct genotype for *S. paratyphi* B or *S. java*.

Analysis of the copy number and chromosomal loci of a *Salmonella*-specific DNA insertion element, IS200 (Lam & Roth, 1983a, b, 1986), has provided useful insights into evolutionary and epidemiological relationships of several *Salmonella* serovars of public health importance (Stanley *et al.*, 1991, 1992, 1993; Ezquerro *et al.*, 1993). The objective of this study was to combine IS200 profiling with 16S rRNA gene profiling to analyse phylogenetic relationships within the *S. paratyphi* B/*S. java* group. We further sought to establish a practicable chromosomal fingerprinting method and a differential genotypic subtyping scheme for this clinically important group of salmonellae.

Methods

Bacterial strains, culture conditions and characteristics. Strains of *Salmonella* used in this study are listed in Table 1. Clinical and environmental isolates of serotype 1,4,[5],12:b:1,2 were from the

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Abbreviations: dTa⁺ and dTa⁻, ability and inability to ferment (+)-tartrate; ET, electrophoretic type; MLEE, multilocus enzyme electrophoresis.

Table 1. *Salmonella* strains and their deduced genotypes

Strain no.	Source/epidemiology	Year of isolation	16S <i>rrn</i> profile	IS200 profile groups (IP) and copy no. (n)
<i>S. paratyphi B</i>				
UB105	Human infant faeces; sporadic	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB4843	Human faeces	1989	<i>Spj</i> -RI	<i>Spj</i> -IP1.1, n = 7
UB226	Human blood/Chile	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB330	Human bile/Egypt	1990	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB478	Human faeces/Portugal	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB1230	Environmental; surface water	1990	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB143	Human blood	1990	<i>Spj</i> -RII	<i>Spj</i> -IP1.2, n = 7
UB1276	Environmental; sewage sludge	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB1450	Environmental; surface water	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB1746	Human faeces	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB3985	Human blood/Spain	1988	<i>Spj</i> -RI	<i>Spj</i> -IP1.3, n = 6
UB2320	Environmental; sewage sludge	1990	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB2654	Environmental; surface water	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB2993	Human blood/Morocco	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB3782	Human faeces	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB3831	Human blood	1989	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB4698	Human faeces	1991	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB4763	Human blood	1989	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB123	(var. Jersey – monophasic)	1992	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
UB124	(var. Jersey – monophasic)	1992	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
NCTC 3176	UK	1930	<i>Spj</i> -RI	<i>Spj</i> -IP1.0, n = 7
<i>S. java</i>				
UB2239*	Environmental/Malaysia	1990	<i>Spj</i> -RI	<i>Spj</i> -IP2.0, n = 8
UB62	Environmental/Indonesia	1990	<i>Spj</i> -RI	<i>Spj</i> -IP2.1, n = 7
UB948	Environmental/Philippines	1991	<i>Spj</i> -RI	<i>Spj</i> -IP2.2, n = 6
UB3936	Human faeces	1991	<i>Spj</i> -RI	<i>Spj</i> -IP2.3, n = 8
UB1925	Environmental/Kenya	1991	<i>Spj</i> -RI	<i>Spj</i> -IP2.4, n = 7
UB5207	Human faeces	1990	<i>Spj</i> -RI	<i>Spj</i> -IP2.5, n = 6
UB480	Human faeces	1990	<i>Spj</i> -RI	<i>Spj</i> -IP2.6, n = 5
UB3276	Human infant faeces; sporadic	1991	<i>Spj</i> -RI	<i>Spj</i> -IP2.2, n = 6
UB4958	Human faeces	1989	<i>Spj</i> -RI	<i>Spj</i> -IP2.6, n = 5
NCTC 5706	Denmark	1937	<i>Spj</i> -RIII	<i>Spj</i> -IP3.0, n = 11
UB1453	Human faeces	1990	<i>Spj</i> -RIV	<i>Spj</i> -IP4.0, n = 6
<i>S. typhimurium</i>				
NCTC 12416	CIP 60.62T = LT2, Demerec	1960	NT	n = 6

NT, Not tested.

* This strain had a delayed positive (+)-tartrate reaction.

recent investigative programme of the Swiss National Reference Laboratory for Foodborne Disease unless otherwise stated. Twenty-two strains were dTa⁻ and ten were dTa⁺ in 1–2 d. One of the dTa⁻ strains (UB2239) became dTa⁺ upon prolonged incubation, as is occasionally the case (Kauffmann, 1966). It should be noted that slime wall production is a general feature only of freshly isolated dTa⁻ strains, and is sometimes a difficult character to assess (Kauffmann, 1966). Strains were grown in nutrient broth/agar for DNA isolation, and purity was checked on blood agar plates. Stock cultures were maintained on Dorset-egg agar slopes.

DNA preparation and hybridization. The presence of plasmid DNA was screened by the method of Kado & Liu (1981). Genomic DNA was extracted from *S. paratyphi B* and *S. java* by the method of Wilson (1987), and 5 µg quantities were digested with one of four enzymes (*Sma*I, *Pst*I, *Pvu*II and *Bgl*II) which lack restriction sites within the IS200 sequence (Gibert *et al.*, 1991; Lam & Roth, 1986). Genomic restriction digests were electrophoresed in 0.7% agarose, and vacuum-blotted (LKB Vacu-gene apparatus) on to Hybond N nylon membrane (Amersham). Hybridization with biotinylated probes was done by standard methods (Sambrook *et al.*, 1989) and filters were stringently

washed (final wash in 0.16 × SSC and 0.1% SDS for 40 min at 60 °C; 1 × SSC is 0.15 M-NaCl plus 0.015 M-trisodium citrate). Homologous bands were visualized colorimetrically with the BluGENE (Gibco-BRL) detection system.

DNA probes. Plasmid pIZ45 was purified by standard methods (Sambrook *et al.*, 1989), and a 300 bp internal fragment of IS200 was amplified from a 620 bp *Pvu*II fragment of pIZ45 by polymerase chain reaction (PCR) with forward and reverse sequencing primers under standard conditions (Innis *et al.*, 1990). The product was subjected to centrifugal ultrafiltration (Millipore Ultrafree-MC; 30,000 NMWL unit), and labelled with 16-dUTP biotin by random primed labelling (Boehringer-Mannheim kit).

A probe internal to the 16S *rrn* gene was prepared by PCR from genomic DNA of *S. paratyphi B* NCTC 3176, employing as primers the sequences 5'-AATTGAAGAGTTTGATCATG-3' and 5'-AGCCATGCAGCACCTGTCTC-3', which represent nucleotides 2–21 and 1040–1059 of the homologous *Escherichia coli* 16S *rrnB* sequence (Brosius *et al.*, 1978). A PCR product of approximately 1000 bp was identified on NuSieve 3:1 agarose (FMC Bio Products) gel, purified with GeneClean (BIO101), and labelled with 16-dUTP biotin as above.

Results

Analysis of 16S *rrn* profiles

Four different patterns of restriction site variation around the 16S *rrn* genes were detected among the 32 strains analysed. Of the restriction enzymes investigated, *Pvu*II was found to be the most suitable for resolving 16S *rrn* profiles, and the number of *Pvu*II bands varied from five to seven (Fig. 1). The great majority of strains (29/32) both of *S. paratyphi B* and of *S. java* exhibited a profile consisting of six *Pvu*II bands sized at 3.5, 4.5, 6.2, 8.0, 8.4 and 9.5 kbp (e.g. NCTC 3176, Fig. 1, track 1). This predominant profile was termed *Spj*-RI. The two examples of *S. paratyphi B* phagovar Jersey, which express only one of the flagellar antigens, shared this predominant profile. Unique 16S *rrn* profiles of *S. paratyphi B* UB143 (Fig. 1, track 4) and of *S. java* UB1453 (Fig. 1, track 3) were termed *Spj*-RII and *Spj*-RIV respectively. In *S. java* NCTC 5706 (Fig. 1, track 2), the 4.5 kbp band of *Spj*-RI was replaced by one of 5.2 kbp, generating a unique profile termed *Spj*-RIII. The notable feature of the 16S *rrn* profiles was that the predominant one was shared by the majority of strains, irrespective of their designation as *S. paratyphi B* or *S. java*.

Fig. 1. 16S *rrn* gene profiles of *S. paratyphi B*/*S. java*. Genomic Southern blot made with *Pvu*II, hybridized with a 1000 bp internal fragment of the 16S *rrn* gene. Track 1 shows NCTC 3176, which has the profile found in 20/21 strains of *S. paratyphi B* and 9/11 of *S. java*. Track 2 shows the unique profile of *S. java* NCTC 5706. Track 3 shows the unique profile of *S. java* UB1453. Track 4 shows the unique profile of *S. paratyphi B* UB143.

Analysis of IS200 profiles

The copy number and loci of IS200 were examined in genomic Southern blots made with *Pst*I (Fig. 2), *Pvu*II or *Bgl*II. Optimum IS200 band resolution was obtained with *Pst*I or *Pvu*II. In the great majority of strains, of both *S. paratyphi B* and *S. java*, common IS200 bands were observed. In *Pvu*II digests (data not shown), all except three strains (*S. java* NCTC 5706, *S. java* UB1453 and *S. paratyphi B* UB3985) contained common IS200 bands of 2.7 and 4.0 kbp. In *Pst*I digests (Fig. 2) there was a large band (approx. 23 kbp) which was likely to be common to all strains. All 21 strains of *S. paratyphi B* and 8/11 strains of *S. java* shared a 10 kbp IS200 band; 20/21 *S. paratyphi B* and 10/11 *S. java* shared a 5.8 kbp IS200 band, whilst all 21 *S. paratyphi B* strains and 6/11 *S. java* shared a 5.0 kbp IS200 band. In summary, there were two conserved *Pvu*II bands or three generally conserved *Pst*I bands. These bands were considered to represent insertion sites present in a common ancestor of the *S. paratyphi B*/*S. java* complex. It should be noted, however, that the 23 kbp band common to all strains and the 20 kbp band common to all but two strains, present some ambiguity due to the limitations of sizing large restriction fragments by gel electrophoresis.

The *S. java* profiles were clearly distinguishable from the *S. paratyphi B* profiles (see Fig. 3*a*). In particular, a *Pst*I band of 3.7 kbp or two *Pvu*II bands (8.4 and 8.8 kbp; data not shown) were unique to all strains of *S. paratyphi B*, and were absent from all strains of *S. java*. There was a major group of related profiles within *S. java* with a 'core pattern' of five bands from 5 to 23 kbp, (Fig. 2, tracks 3–8 and 13), and all *S. paratyphi B* strains had a 'core profile' of six IS200 bands sized from 3.7 to 23 kbp. Strain UB2239 was exceptional in that it had originally been placed within the *S. paratyphi B* set on the basis of a negative (+)-tartrate reaction in the standard time. Its IS200 profile (Fig. 2, track 13), however, placed it among the *S. java* isolates. The strain was therefore re-examined, revealing a delayed (+)-tartrate positive reaction, and also the absence of a slime wall, redefining the phenotype as that of *S. java*.

One IS200 profile (e.g. UB3831: Fig. 2, track 9) was conserved among 18/22 *S. paratyphi B* strains, and was invariably linked with the 16S *rrn* profile *Spj*-RI. It was shared by NCTC 3176 (an isolate from 1930) and contemporary isolates from diverse sources (including human isolates from faeces, blood and bile, environmental isolates from surface water or sewage and the two examples of the monophasic phagovar 'Jersey'). This conserved profile occurred irrespective of the geographical origin of the isolates. It had a copy number of seven, one copy being located on a characteristic large *Pst*I fragment of about 30 kbp, and was termed *Spj*-IP1.0.

Fig. 2. IS200 hybridization with genomic DNA of *S. paratyphi* B and *S. java*. Genomic Southern blot (*Pst*I) hybridized with internal fragment of IS200. Tracks 1–8 contain strains with different IS200 profiles found among regular dTa⁺ *S. java* strains (1, *S. java* NCTC 5706; 2, UB1453; 3, UB62; 4, UB948; 5, UB3936; 6, UB1925; 7, UB5207; 8, UB480). Tracks 9–12 contain strains with four different IS200 profiles found among dTa[−] *S. paratyphi* B strains (9, *S. paratyphi* B UB3831; 10, UB4843; 11, UB3985; 12, UB143). Track 13 contains UB2239, an *S. java* strain with an atypical delayed-positive D-tartrate reaction. On the left and right hand sides are shown internal controls: *Hind*III fragments of phage λ as molecular size markers, and the positions of the six IS200 bands found in *S. typhimurium* NCTC 12416.

Three unusual IS200 profiles were found among the *S. paratyphi* B strains. Their copy numbers were six or seven, and all lacked the 30 kbp *Pst*I band. These profiles, which were termed *Spj*-IP1.1 to *Spj*-IP1.3, were those of strains UB4843, UB3985 and UB143 (Fig. 2, tracks 10–12). *Spj*-IP1.1 and *Spj*-IP1.3 were linked to the 16S *rrn* profile *Spj*-RI. *Spj*-IP1.2 was linked to 16S *rrn* profile *Spj*-RII.

In *S. java*, IS200 copy number varied from five (UB480 and UB4958) to eleven (NCTC 5706). A greater variety of IS200 profiles was found in *S. java* than in *S. paratyphi* B: nine profiles were found among only eleven strains. The predominant profile *Spj*-IP1.0 of *S. paratyphi* B was not found in *S. java*, although 9/11 *S. java* strains shared the predominant 16S *rrn* profile (*Spj*-RI) of *S. paratyphi* B. Two IS200 profiles of *S. java* were notably less related either to each other, or to the remaining seven profiles. They occurred in NCTC 5706 and UB1453 (Fig. 2). These atypical IS200 profiles were linked with unique 16S *rrn* profiles, *Spj*-RIII and *Spj*-RIV, and were termed *Spj*-IP3.0 and *Spj*-IP4.0. Nonetheless these strains had at least one conserved IS200 locus, carried on a 10 kbp *Pst*I fragment (Fig. 2), a *Pvu*II fragment of 4 kbp or a *Bgl*II fragment of about 17 kbp (data not shown). The seven remaining IS200 profiles of *S. java* (*Spj*-IP2.0 to *Spj*-IP2.6) were related by combinations of several

common bands, indicative of conserved ancestral insertion loci. None of them carried the 3.7 kbp band unique to *S. paratyphi* B.

Plasmid DNA was not detected in any strain, and it was therefore concluded that all insertions of IS200 were chromosomal. These chromosomal IS200 bands were compared between all strains, using a computer program to cluster similarities hierarchically in a rooted dendrogram (Fig. 3b) which represents the inter-strain relationships.

Discussion

The first probe used to characterize rRNA gene restriction patterns, or 'ribotypes', was *E. coli* rRNA (Grimont & Grimont, 1986) and this probe detected two, four and six 'ribotypes' respectively in *Salmonella typhimurium*, *S. reading* and *S. senftenberg* (Esteban *et al.*, 1993). The cloned *E. coli* *rrn* operon (Brosius *et al.*, 1978) probe similarly detected multiple ribotypes of *S. typhi* (Pang *et al.*, 1992). An intragenic 16S *rrn* probe fragment generated by PCR detected three well-defined 16S *rrn* profiles within *S. bovis* (Ezquerro *et al.*, 1993). Among four 16S *rrn* profiles found in serotype 1,4,[5],12:b:1,2, one, *Spj*-RI, was predominant, as has previously been shown for other serotypes (Ezquerro *et*

Fig. 3. IS200 profile groups in *S. paratyphi B*/*S. java* and derived phylogenetic relationships. (a) Diagrammatic representation of IS200 profile groups. The first four (left to right) *Pst*I profiles were those of *S. paratyphi B*. The prevalence of these profiles was as follows: *Spj*-IP1.0 (18/21 strains), *Spj*-IP1.1, *Spj*-IP1.2, *Spj*-IP1.3 (all 1/21 strains). The largest band in *Spj*-IP1.0 was not invariably found and is shown by a broken line. The next nine profiles were those of *S. java*, and their prevalence was: *Spj*-IP2.0 (1/11 strains), *Spj*-IP2.1 (1/11 strains), *Spj*-IP2.2 (2/11 strains), *Spj*-IP2.3, *Spj*-IP2.4, *Spj*-IP2.5 (1/11 strains), *Spj*-IP2.6 (2/11 strains), *Spj*-IP3.0 and *Spj*-IP4.0 (1/11 strains). The profiles *Spj*-IP3.0 and *Spj*-IP4.0 had fewest IS200 bands in common with others, and the two strains with these profiles also had unique 16S *rrn* profiles (see Fig. 1). (b) Phylogenetic relationships based on IS200 profiling. The dendrogram was constructed by hierarchical clustering of inter-strain similarities, based on unweighted pair group method analysis with arithmetic averages (UPGMA). The band of about 23 kbp was treated as conserved for this analysis.

al., 1993; Esteban *et al.*, 1993). It was distinct from the major profile of *S. typhimurium*, another Group B serovar, but not exclusive to *S. paratyphi B*/*S. java*, since we have observed it in nine serovars including *S. dublin* and *S. enteritidis* (J. Stanley, N. Powell & C. S. Jones, unpublished). We conclude that 16S *rrn* gene profiles may differ within a serovar, but are sometimes common to many serovars. A conclusion which might be drawn from this study is that a single 'randomly selected' trait such as rRNA gene profiling may be an inadequate

marker for the whole genome, since variables which do not alter that trait may drastically affect virulence.

In *S. paratyphi B*/*S. java*, the profiles of the insertion element IS200 were directly related to, but more differential than, the 16S *rrn* profiles. They demonstrated that the majority of strains, irrespective of their dTa^{+/−} status, belonged to one phylogenetic complex. Combinations of three loci (*Pst*I bands of 5.0, 5.8 and 10 kbp) were conserved in most dTa⁺ and dTa[−] strains, consistent with common chromosomal genetic origin for *S. paratyphi B* and *S. java*. These three IS200 bands were not found in *S. typhimurium*, *S. heidelberg*, *S. typhi*, *S. enteritidis*, *S. panama*, nor in 20 other serovars analysed in our laboratory (data not shown), and we concluded that the presence of one or other of them is characteristic of *S. paratyphi B*/*S. java*. MLEE studies (Selander *et al.*, 1990a, b) previously indicated the common ancestry of *S. paratyphi B* and *S. java*, and two other features of this study also parallel the MLEE analysis. Firstly, a predominant clone (*Spj*-IP1.0) of *S. paratyphi B* was detected by IS200 profiling, just as a predominant ET clone of *S. paratyphi B* was detected by MLEE. Whilst the total number of strains in the present study was relatively small, and many were from Switzerland, over a quarter of *Spj*-IP1.0 strains originated in countries as diverse as Chile and Egypt, consistent with the global distribution of this clone. Secondly, the MLEE study found greater genetic variation in *S. java* than in *S. paratyphi B*. In this study, too, nine IS200 profiles were detected among only eleven strains of *S. java*, as opposed to four profiles among the twenty-one strains of *S. paratyphi B*. We hypothesize that since *S. java* has many animal, as well as human hosts, a variety of selection pressures (adaptation to different hosts) exists for the evolution of genotypic diversity. For example, in Indochina, where *S. java* is frequently isolated from human salmonellosis, rodents, fish and shellfish are implicated as reservoirs of zoonotic infection (Nguyen *et al.*, 1975).

The IS200 profiles of two *S. java* strains, NCTC 5706 and UB1453, each showed many unique features. They were the least-related members of the phylogenetic complex shown in Fig. 3(b) and their 16S *rrn* profiles were correspondingly unique. A good example of the capacity of IS200 profiling to resolve ambiguity in phenotypic typing was provided by strain UB2239, which had been identified as *S. paratyphi B* on the basis of a negative (+)-tartrate reaction under standard test conditions, but whose IS200 profile (Fig. 2) correctly grouped it as *S. java*. No distinct IS200 profile could, however, be assigned to those *S. paratyphi B* isolates in this study which were from septicæmic (blood) infections. Four of six such isolates belonged to the predominant profile *Spj*-IP1.0. This analysis could not therefore determine whether these septicæmias were due

to bacterial genotype or host factors. Finally, it should be noted that extrachromosomal DNA was not found in any strain. Plasmid profiling is therefore not a potential subtyping method, and since IS200 profiles are chromosomal fingerprints, they are particularly valuable for the epidemiological analysis of this serovar.

Comparison of the relative sensitivity of MLEE and IS profiling was made by Sawyer *et al.* (1987), who analysed the IS bands of six *E. coli* elements. Identical ETs of *E. coli* (Selander *et al.*, 1986) differed in over half their IS bands. In *Salmonella*, the resolution of chromosomal genotype by IS200 profiling is precise and discriminatory when a serovar contains moderately high copy numbers of the element, as does *S. heidelberg* (Stanley *et al.*, 1992) or *S. typhimurium* (Stanley *et al.*, 1993). Good resolution was obtained between and within the chromosomes of *S. paratyphi B* and *S. java* in this study. Whereas no distinguishing features had been found to separate them by MLEE analysis (Selander *et al.*, 1990*a, b*), distinct genotypes could be assigned to them, for the first time, by IS200 profiling. Other advantages of IS200 profiles are that they can be generated with a single defined probe on the basis of common techniques, displayed photographically or graphically as markers of genotype, and employed in a band-matching database for inter-laboratory comparison. IS200 profiling demonstrates clearly the complementarity and continuity between the phylogenetics and genotypic typing of *Salmonella*.

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