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Molecular Subtyping Scheme for *Salmonella panama*

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We describe a genotyping scheme for *Salmonella panama*. Defined probes specific for the 16S rRNA gene and the DNA insertion element IS*200* were generated by PCR from *S. panama* and were used to probe genomic Southern blots made with enzymes selected to cut within and outside the probed sequences. Plasmid profiles were determined. The typeability and discriminatory power of the individual methods were compared. Ribotyping with 16S rRNA gene probe alone was slightly more discriminatory than phage typing, but unlike the latter, ribotyping was able to type all strains. IS*200* profiling was the single most discriminatory method for *S. panama*, having an index of discrimination (*D*) of 0.8 and 100% typeability. Plasmid profiling, which had moderate discriminatory power but only 50% typeability, was valuable as an adjunct technique. The use of all three methods together or simply the combination of IS*200* profiling with the two most discriminatory enzymes and plasmid profiling yielded a molecular typing scheme whose discriminatory power ($D = 0.97$) approached the maximum theoretical value. This should prove both useful and robust for epidemiological investigations of *S. panama*.

Salmonella panama, a member of O serogroup D_1, is consistently one of the most commonly isolated serotypes from humans with nontyphoidal salmonellosis in the United Kingdom and other countries (2, 23). The serotype generally causes gastroenteritis, but it is one of several serotypes that tend to cause disease more invasive than that caused by other serotypes (29) and is associated with bacteremias and meningitis (19) as well as pelvic inflammatory disease (18).

An important reservoir of *S. panama* is the porcine host. *S. panama* had not been isolated from humans in the United Kingdom before 1939, but the feeding of contaminated dried egg to pigs led to the infection of these hosts, and since 1944 it has been recovered repeatedly from humans and porcine products (30). *S. panama* has been among the predominant serotypes isolated from healthy or scouring pigs, uncooked sausage meat, and butchers' shops (6, 9, 14, 15).

Multilocus enzyme electrophoresis detected 13 electrophoretic types of *S. panama* which are related to those of *Salmonella miami*, another group D_1 serotype, with which it probably coevolved (26). Phenotypic subtyping of *S. panama* for epidemiological purposes has been made by the scheme of Guinée, which distinguishes eight phage types (types A to H) and a residual group of strains (11). *S. panama* strains are reported to carry a heterogeneous population of plasmids (13), but it was 1 of 11 serotypes found not to harbor large virulence plasmids (24) carrying *spv* (*Salmonella* plasmid virulence [12]) genes.

The mobile genetic element IS*200* is the smallest known DNA insertion sequence and is found primarily in *Salmonella* spp. (20). Restriction fragment length polymorphism in and around IS*200* loci has proved to be a valuable tool for molecular epidemiological subtyping of *S. typhimurium* (4, 5, 27). No such method has been reported for *S. panama*; in the present report we describe a genotypic subtyping scheme for the serotype, combining 16S ribotyping with IS*200* profiling (3, 27, 28) and plasmid profiling to maximize typeability (the ability of a test to provide an unambiguous result for each isolate examined) and discriminatory power (21).

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MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains of *S. panama* designated by UB (University of Berne) markers were from the Collection of the Swiss National Reference Laboratory for Foodborne Disease, University of Berne, and are listed in order of accession in Table 1. The National Collection of Type Cultures (NCTC; London, United Kingdom) type strain and the reference strain of the principal multilocus enzyme electropherotype, Pn1 (7), were included in the study. Stock cultures were maintained as lyophilized cultures and on Dorset egg agar slopes at room temperature for the duration of the study. Strains were grown in nutrient broth for DNA isolation, and purity was checked on blood agar plates.

Nucleic acid techniques. A 1,500-bp probe was generated by PCR from the genomic DNA of *S. panama* NCTC 5774 with the primers 5′-AAGAGTTTGA TCCTGGCTCAG-3′ and 5′-GGTTACCTTGTTACGACTT-3′, corresponding to nucleotides 7 to 27 and 1492 to 1510, respectively, of the *Escherichia coli* 16S rRNA gene sequence (8). IS*200* profiling was made with a probe consisting of a 692-bp PCR amplicon internal to the element that was produced as described previously (4). PCR products were separated from primers with Gene Clean (Bio 101 Inc., La Jolla, Calif.) and were labelled with 16-dUTP-biotin by using a random-primed labelling kit (Boehringer-Mannheim Biochemica GmbH, Mannheim, Germany). Genomic DNA extraction, agarose gel electrophoresis, vacuum blotting, and hybridization conditions were as described previously (27). Nylon membrane filters were washed under stringent conditions in 0.16× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate)–0.1% sodium dodecyl sulfate. The presence of plasmids was screened by the method of Kado and Liu (17).

RESULTS

Plasmid profiles and inapplicability of PFGE. Fifteen of 30 strains contained plasmids (Table 1), and plasmids of 24, 4, 2.4, and 1.4 MDa were common to many of them. The plasmid-free state was scored as profile PP0, and eight other plasmid profiles could be distinguished (Table 1). In a Southern blot with plasmid DNAs of all strains, no hybridization was found with IS*200*.

Standard methods of DNA preparation for pulsed-field gel electrophoresis (3, 22) were found to be nonfunctional for *S. panama* because of DNA degradation upon the addition of endonuclease buffer. Formalin treatment (10) was applied to

TABLE 1. Epidemiology and genotypes of *S. panama* strains

Strain no.	Source[a]	16S rRNA gene profiles[b]				IS*200* profile (no. of bands)[b,c]				Size of plasmid bands (MDa)	Plasmid profile
		*Pvu*II	*Hin*dIII	*Pst*I	*Mlu*I	*Pvu*II	*Eco*RI	*Pst*I	*Eco*RV		
NCTC 5774	1934, Copenhagen	P4	H1	P′2	M6	P11 (7)	E11 (16)	P′10 (7)	E′10 (15)		0
NCTC 6852	1944, Kentucky	P5	H3	P′2	M7	P12 (7)	E12 (18)	P′11 (7)	E′11 (16)		0
UB2-83	Human, fecal, LTE	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	7, 5	7
UB40-83	Human, fecal, LTO	P1	H1	P′1	M1	P4 (8)[d]	E4 (15)	P′4 (8)[d]	E′3 (15)		0
SARB 39	Reference 7	P2	H2	P′2	M3	P3 (7)	E3 (13)	P′3 (7)	E′2 (13)		0
UB121-83	Human, fecal, LTG	P1	H1	P′1	M1	P1 (8)	E1 (15)	P′1 (8)	E′1 (14)		0
UB243-83	Lake water (Neuchâtel), LTA	P1	H1	P′1	M1	P2 (7)	E2 (13)	P′2 (7)	E′1 (14)		0
UB529-83	Environmental, Norway, LTA	P1	H1	P′1	M1	P2 (7)	E2 (13)	P′2 (7)	E′1 (14)		0
UB585-83	Human, fecal, LTG	P1	H1	P′1	M1	P1 (8)	E1 (15)	P′1 (8)	E′1 (14)	24	1
UB864-83	Human, fecal, LTB	P2	H3	P′2	M2	P5 (7)	E5 (15)	P′5 (7)	E′4 (11)	24, 1	3
UB1342-83	Human, bile, LTG	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	24, 1.5	4
UB2050-83	Human, fecal, LTA	P1	H4	P′3	M4	P6 (9)	E6 (15)	P′6 (9)	E′5 (15)	24	1
UB2720-93	River water (Birs)	P1	H1	P′1	M1	P7 (9)	E7 (16)	P′7 (9)	E′6 (15)	24, 4, 2.4	5
UB2982-93	Egg products, Norway	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	30, 24	2
UB3536-93	Stream water (Rhine)	P1	H1	P′1	M1	P8 (8)	E8 (16)	P′8 (9)	E′7 (15)		0
UB3607-93	Dairy product, Mexico	P2	H2	P′2	M2	P9 (9)	E9 (19)	P′9 (9)	E′8 (14)		0
UB4708-93	Meat	P3	H1	P′1	M1	P2 (7)	E2 (13)	P′2 (7)	E′1 (14)		0
UB4862-83	Human, pus, LT C	P1	H1	P′1	M1	P2 (7)	E2 (14)	P′2 (7)	E′1 (14)		0
UB4978-91	Human, blood	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)		0
UB5143-93	Human, not specified	P2	H2	P′2	M2	P3 (7)	E3 (13)	P′3 (7)	E′2 (13)		0
UB5205-91	Human, fecal	P1	H1	P′1	M5	P10 (7)	E10 (13)	P′2 (7)	E′9 (13)		0
UB5618-89	Case cluster, human, fecal	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	4, 2.4	8
REF 262	Reference strain LTA	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	24	1
REF 263	Reference strain LTB	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	24	1
SSZ 741	Reference strain LTC	P1	H1	P′1	M1	P2 (7)	E2 (14)	P′2 (7)	E′1 (14)	24	1
REF 264	Reference strain LTD	P1	H1	P′1	M1	P2 (7)	E2 (14)	P′2 (8)	E′1 (14)		0
REF 265	Reference strain LTE	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	4, 2.4	8
SSZ 251	Reference strain LTF	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	4, 2.4, 1.5, 0.8	9
REF 266	Reference strain LTG	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	20, 2.4, 1.5	6
REF 267	Reference strain LTH	P1	H1	P′1	M1	P1 (8)	E1 (14)	P′1 (8)	E′1 (14)	24	1

[a] The phage type, if known, is also given. LTA through LTH are phage types of *S. panama* (11). All human isolates were from sporadic cases in Switzerland unless stated otherwise.
[b] Arabic numbers following the enzyme code (P, PvuII; H, *Hin*dIII; P′, *Pst*I; M, *Mlu*I) indicate different patterns of hybridization with the probe.
[c] P, *Pvu*II; E, *Eco*RI; P′, *Pst*I; E′, *Eco*RV.
[d] Certain faint bands were discounted; see legend to Fig. 2.

DNA-containing agarose blocks to overcome nuclease activity, but this was also unsuccessful. Control DNAs of *Salmonella brandenburg* (3) and *Salmonella typhimurium* yielded intact DNA suitable for restriction enzyme digestion.

16S rRNA gene polymorphism. A 1,500-bp (internal) fragment of the 16S rRNA gene was amplified from *S. panama* NCTC 5774 and was used to probe genomic Southern blots made with four enzymes, *Pvu*II, *Hin*dIII, *Pst*I, and *Mlu*I. Six bands ranging from 3.5 to ≈17 kbp in size were detected in *Pvu*II blots (Fig. 1, lanes 1 to 5), 6 bands from 6 to ≈30 kbp were detected in *Hin*dIII blots, 6 bands from 8 to ≈30 kbp were detected in *Pst*I blots (data not shown), and 13 bands from 1.7 to ≈25 kbp were detected in *Mlu*I blots (Fig. 1, lanes 7 to 15). The resulting 16S rRNA gene polymorphisms were assigned the codes P, H, P′, and M. Five 16S rRNA gene polymorphisms were detected with *Pvu*II, four were detected with *Hin*dIII, three were detected with *Pst*I, and seven were detected with *Mlu*I; *Mlu*I was the only one of the four enzymes to cut within the probed nucleotide sequence. The 16S gene polymorphisms obtained for the two most discriminatory enzymes, *Pvu*II and *Mlu*I, are shown in Fig. 1. The discriminatory power of the method was assessed by calculating numerical indices of discrimination (*D* values [16]); they were 0.41 for *Pvu*II, 0.36 for *Hin*dIII, 0.38 for *Pst*I, and 0.46 for *Mlu*I. Combined ribotyping with the two most discriminatory enzymes (Fig. 1) yielded a *D* value of 0.51. Combined ribotyping with all four enzymes yielded a *D* value of 0.52.

Reference strains of the eight phage types of *S. panama* (11) exhibited the same combined 16S ribotype (P1 H1 P′1 M1). However, while the ribotype of the LTA reference strain (P1 H1 P′1 M1) was shared by two environmental LTA isolates (UB243-83 and UB529-93), a third LTA isolate from a human (UB2050-83) had ribotype P1 H4 P′3 M4.

In general two 16S rRNA gene polymorphisms were found in multiple strains (shaded regions in Table 2). Seven other polymorphisms were observed only once in the set.

IS*200* profiling. IS*200* profiles were generated from genomic Southern blots made with two enzymes which do not cut within the sequence of the element (*Pvu*II [P] and *Pst*I [P′]) and with two enzymes which cut once within the element (*Eco*RI [E] and *Eco*RV [E′]). In *Pvu*II profiles (Fig. 2A) seven to nine IS*200* bands were detected. They were between 1.7 and ≈30 kbp, and all strains carried four conserved IS*200* bands of 6, 10, ≈26, and ≈30 kbp. In *Pst*I profiles (Fig. 2B) there were seven to nine IS*200* bands from 4 to ≈28 kbp in size. As with *Pvu*II, four IS*200* bands (6.5, 8.8, 13, and 23 kbp) were conserved between all *S. panama* strains. In *Eco*RI profiles (Fig. 3, lanes 1 to 12) the numbers of IS*200* bands ranged from 13 to 19, and they were sized between 0.5 and ≈30 kbp. At least eight bands (1.1, 1.7, 2.0, 2.1, 2.2, 2.6, 6.3, and 9.3 kbp) were conserved between all strains. In *Eco*RV profiles (Fig. 3, lanes 14 to 24) 13 to 16 IS*200* bands were detected, and 8 of these bands (sized 0.7, 0.9, 1.2, 1.6, 3.0, 5.4, 8.0, and 9.4 kbp) were conserved. In general the profiles were not ambiguous, but faint bands in

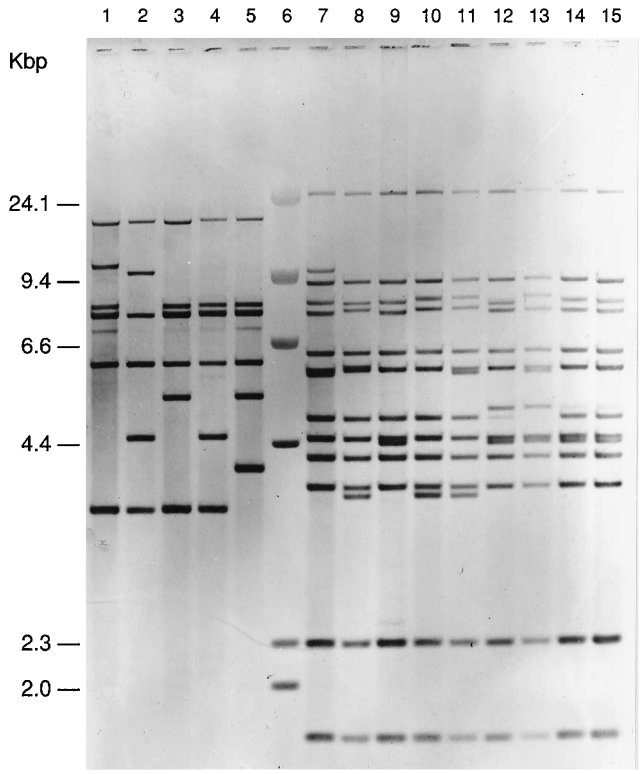

FIG. 1. 16S rRNA gene polymorphisms in *S. panama*. Genomic Southern blot made with *Pvu*II (P) (lanes 1 to 5) or *Mlu*I (M) (lanes 7 to 15) probed with a 1,500-bp PCR amplicon internal to the 16S rRNA gene. Lanes: 1, strain NCTC 5774 (P4 of Table 1); 2, NCTC 6852 (P5); 3, UB3536 (P1); 4, SARB 39 (P2); 5, UB4708 (P3); 6, bacteriophage lambda DNA digested with *Hin*dIII; 7, NCTC 5774 (M6 of Table 1); 8, NCTC 6852 (M7); 9, UB2720 (M1); 10, SARB 39 (M3); 11, UB5143 (M2); 12, UB2050 (M4); 13, UB5205 (M5); 14, UB40 (M1); 15, REF 262 (M1).

some digests were not counted (cf. strain UB40 in Fig. 2A and B) if the other enzyme digests (Fig. 3) concurred to give the probable copy number of IS*200*.

The 11 IS*200* profiles found with *Pst*I and *Eco*RV were termed P′1 to P′11 and E′1 to E′11, respectively. The 12 profiles found with *Pvu*II and *Eco*RI were termed P1 to P12 and E1 to E12, respectively. The respective *D* values were calculated and are as follows: *Pst*I, 0.77; *Eco*RV, 0.61; *Pvu*II, 0.78; and *Eco*RI, 0.78. Combined IS*200* profiling with the two most discriminatory enzymes yielded a *D* value of 0.78, and combined IS*200* profiling with all four enzymes yielded a *D* value of 0.8.

Analysis of combined genotypes. Table 2 gives a summary of all *S. panama* genotypes. Of the nine 16S rRNA gene polymorphisms, two contained multiple strains and seven were specific to one strain in the set. All 16S rRNA gene polymorphisms had unique corresponding IS*200* profiles (cf. Table 2). The first 16S rRNA gene type (P1 H1 P′1 M1) was composed of 21 strains, which could be separated into five IS*200* profiles. Two of these five IS*200* profiles (P1 E1 P′1 E′1 and P2 E2 P′2 E′1) contained multiple strains (13 and 5, respectively; Table 2); the other three were specific to individual strains in the set (strains UB40, UB2720, and UB3536). It was noteworthy that the most abundant IS*200* profile type (Fig. 2A and B, lanes 1) differed from the second most abundant profile type (Fig. 2A and B, lanes 2) by a single transposition event. It could be subdivided into eight plasmid profiles. On the other hand,

TABLE 2. *S. panama* strains and their genotypes

16S ribotype	IS*200* profile	Plasmid profile	No. of strains
P1 H1 P′1 M1	P1 E1 P′1 E′1	1	4
P1 H1 P′1 M1	P1 E1 P′1 E′1	0	2
P1 H1 P′1 M1	P1 E1 P′1 E′1	2	1
P1 H1 P′1 M1	P1 E1 P′1 E′1	4	1
P1 H1 P′1 M1	P1 E1 P′1 E′1	6	1
P1 H1 P′1 M1	P1 E1 P′1 E′1	7	1
P1 H1 P′1 M1	P1 E1 P′1 E′1	8	2
P1 H1 P′1 M1	P1 E1 P′1 E′1	9	1
P1 H1 P′1 M1	P2 E2 P′2 E′1	0	4
P1 H1 P′1 M1	P2 E2 P′2 E′1	1	1
P1 H1 P′1 M1	P4 E4 P′4 E′3	0	1
P1 H1 P′1 M1	P7 E7 P′7 E′6	5	1
P1 H1 P′1 M1	P8 E8 P′8 E′7	0	1
P2 H2 P′2 M2	P9 E9 P′9 E′8	0	1
P2 H2 P′2 M2	P3 E3 P′3 E′2	0	1
P2 H3 P′2 M2	P5 E5 P′5 E′4	3	1
P1 H4 P′3 M4	P6 E6 P′6 E′5	1	1
P4 H1 P′2 M6	P11 E11 P′10 E′10	0	1
P5 H3 P′2 M7	P12 E12 P′11 E′11	0	1
P2 H2 P′2 M3	P3 E3 P′3 E′2	0	1
P3 H1 P′1 M1	P2 E2 P′2 E′1	0	1
P1 H1 P′1 M5	P10 E10 P′2 E′9	0	1

within the second most abundant IS*200* profile type (P2 E2 P′2 E′1), four of the five strains lacked any plasmid DNA (Table 2).

The second 16S rRNA gene type (P2 H2 P′2 M2) consisted of two strains (UB3607 and UB5143), each having a corresponding unique IS*200* profile (Fig. 2A and B and Fig. 3) and each lacking plasmids. The remaining seven 16S rRNA gene polymorphisms were found only in one strain; each had a corresponding unique IS*200* profile (Table 2), and only two of them contained plasmid DNA. By combining the results from 16S gene polymorphisms, IS*200* profiles, and plasmid profiles or by combining IS*200* profiles with the two most discriminatory enzymes and plasmid profiles, a total of 22 genotypes could be distinguished. The *D* value in either case was 0.97.

DISCUSSION

The phage types of *S. panama* could be split up by genotyping methods, applied individually or in combination. For example the strains of phage type LTA (REF 262, UB243, UB529, UB2050) could be further differentiated by their 16S rRNA gene polymorphisms alone (three strains with type P1 H1 P′1 M1 and one strain with type P1 H4 P′3 M4), by IS*200* profiling alone (three strains with profile P2 E2 P′2 E′1 and one strain with profile P6 E6 P′6 E′5), by plasmid profiling alone (either PP1 or PP0), or by the combined methods (three genotypes; cf. Table 2). The two strains of phage type LTB (REF 263 and UB864) differed by all three molecular methods, while the LTC, LTE, and LTG strains differed only in their plasmid profiles. Phage typing (11) distinguishes eight types

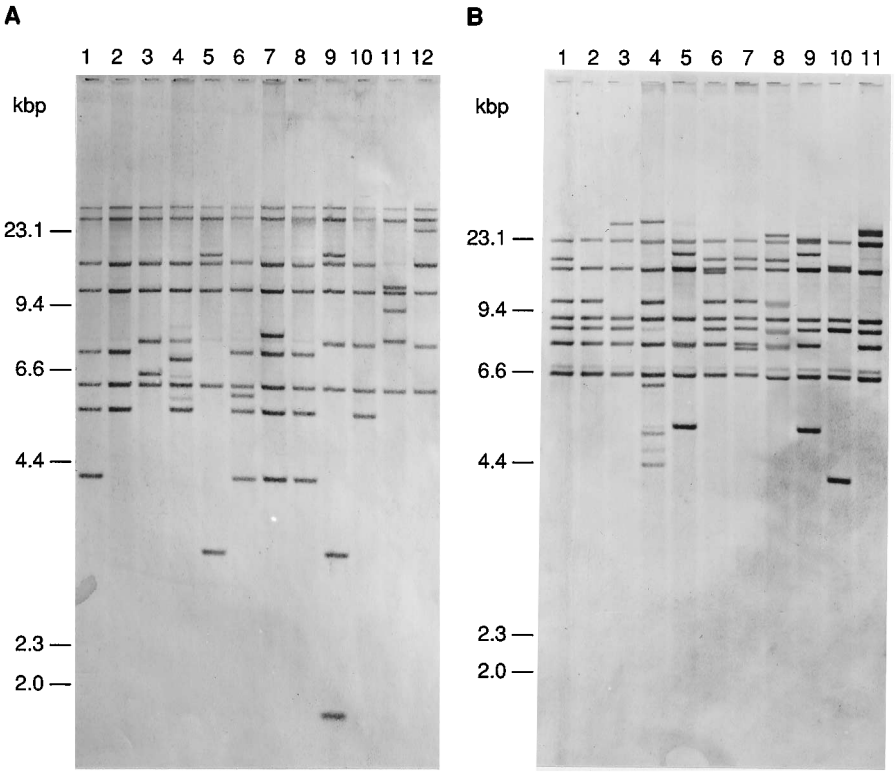

FIG. 2. IS*200* profiles in *S. panama*. Genomic Southern blots made with *Pvu*II (P) (A) or *Pst*I (P′) (B). Blots were probed with a 692-bp PCR amplicon internal to the IS*200* sequence. (A) Lanes: 1, strain REF 266 (P1 of Table 1); 2, SSZ 741 (P2); 3, SARB 39, (P3); 4, UB40 (P4); 5, UB864 (P5); 6, UB2050 (P6); 7, UB2720 (P7); 8, UB3536 (P8); 9, 3607 (P9); 10, UB5205 (P10); 11, NCTC 5774 (P11); 12, NCTC 6852 (P12). (B) Lanes: 1, strain REF 266 (P′1 of Table 1); 2, SSZ 741 (P′2); 3, SARB 39, (P′3); 4, UB40 (P′4); 5, UB864 (P′5); 6, UB2050 (P′6); 7, UB2720 (P′7); 8, UB3536 (P′8); 9, UB3607 (P′9); 10, NCTC 5774 (P′10); 11, NCTC 6852 (P′11). Faint bands such as those seen in strain UB40 (panel A, lane 4, at 5.8, 6.3, and 7.9 kbp; panel B at 4.6, 5.1, and 8.2 kbp) were discounted since other digests (e.g., Fig. 3, lane 4) were compared to confirm the number of IS*200* bands.

within *S. panama*, but its residual group of nontypeable strains limits the usefulness of the method. It is clear that molecular genotyping can yield more discriminatory and more comprehensive subtyping of strains of this serotype.

Plasmid profiling constituted a useful adjunct to the other methods, which address chromosomal DNA. However, it was inadequate as a sole subtyping method, since only half of the strains carried plasmids and could not be further differentiated by this approach. Defining the plasmid-free state as a profile (PP0), a theoretical *D* value of 0.72 was obtained, but typeability could not exceed 50%. Combined 16S rRNA gene typing with four enzymes was somewhat more discriminatory than phage typing, yielding a *D* value of 0.52; however, its typeability was 100%, a factor which has been cited as a general advantage of molecular typing (1).

The technique exhibiting both 100% typeability and the best *D* value for any single method, 0.8, was IS*200* profiling. As shown with *S. typhimurium*, this method yields information of evolutionary as well as epidemiological significance (27). As in the latter case and that of *Salmonella heidelberg* (28), serotype-specific IS*200* loci were detected in *S. panama*. By comparison of digests with enzymes cutting within or outside the sequence of the element, it was established that four of the seven to nine IS*200* loci were conserved among all *S. panama* strains. We have previously described the identification of errors in serotyping (5) by recognition of conserved IS*200* sites, a feature of various *Salmonella* serotypes examined to date (3, 27, 28). Future data analysis programs could be designed to first recognize the serotype and then to subtype the isolates within it on the basis of IS*200* profile.

IS*200* profiling was first described as a tool of molecular epidemiology in relation to *S. heidelberg* and *S. typhimurium* (4, 5, 27, 28) in which only enzymes that cut outside the element were used. In the present study we extended this approach by using enzymes which cut within IS*200* to complete the profiles. If an individual genotyping method is sought for *S. panama*, IS*200* profiling is the most appropriate one in terms of both typeability (21) and discriminatory power (21). This reflects the robustness of the DNA extraction method and the relatively high copy number of the element in the serotype. If IS*200* profiling with the two most discriminatory enzymes is used in conjunction with plasmid profiling, a very high *D* value (0.97) is obtained. Although the utility of pulsed-field gel electrophoresis (22) has been demonstrated for several serotypes (3, 25), it was not found to be applicable to *S. panama* because of intrinsic nuclease activity. This is a significant limitation to the use of pulsed-field gel electrophoresis for some *Salmonella* serotypes.

The present study demonstrated that the discriminatory power of genotyping can be increased in a structured manner by genome digestion with several selected enzymes, combining the restriction fragment length polymorphisms obtained with IS*200* or additionally with 16S rRNA gene probes. When extrachromosomal DNA is also resolved by plasmid profiling, one arrives at a numerical index of discriminatory power (0.97)

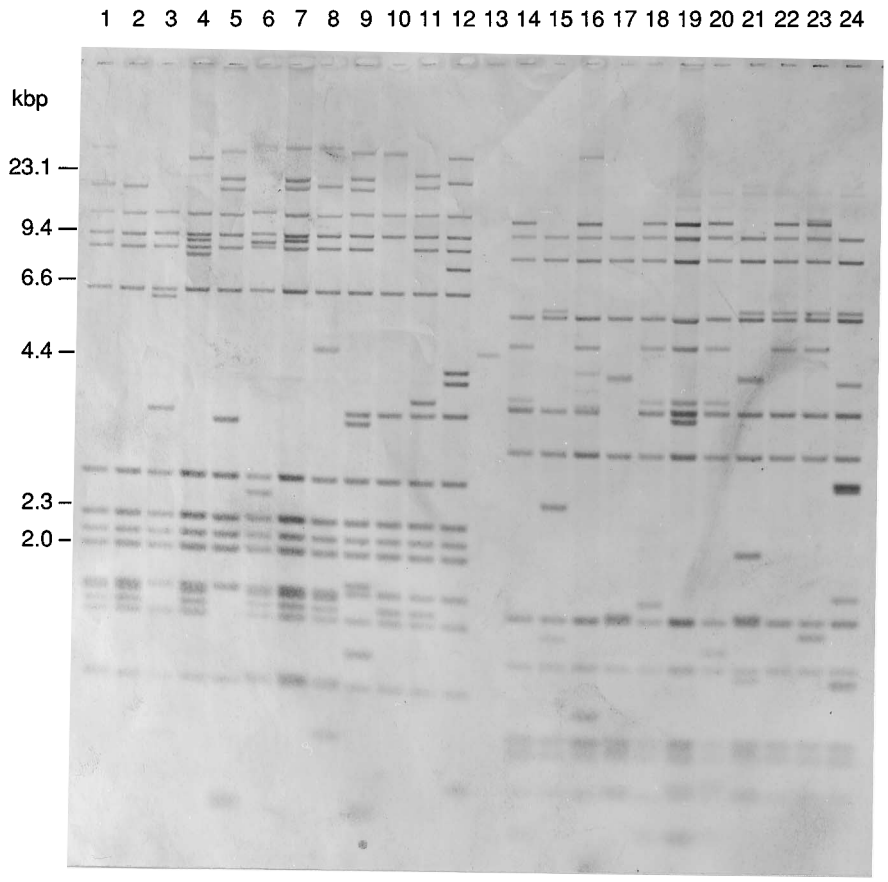

FIG. 3. IS*200* profiles in *S. panama*. Genomic Southern blot made with *Eco*RI (E) or *Eco*RV (E′) and probed as described in the legend to Fig. 2. Lanes: 1, strain REF 266 (E1 of Table 1); 2, SSZ 741 (E2); 3, SARB 39, (E3); 4, UB40 (E4); 5, UB864 (E5); 6, UB2050 (E6); 7, UB2720 (E7); 8, UB3536 (E8); 9, UB3607 (E9); 10, UB5205 (E10); 11, NCTC 5774 (E11); 12, NCTC 6852 (E12); 13, bacteriophage lambda DNA digested with *Hin*dIII; 14, REF 266 (E′1 of Table 1); 15, SARB 39 (E′2); 16, UB40 (E′3); 17, UB864 (E′4); 18, UB2050 (E′5); 19, UB2720 (E′6); 20, UB3536 (E′7); 21, UB3607 (E′8); 22, UB5205 (E′9); 23, NCTC 5774 (E′10); 24, NCTC 6852 (E′11). Hybridization to the 4.4-kbp band of the lambda marker (lane 13) is nonspecific and is probably due to *chl* sequences.

which approaches the theoretical maximum (1.0 [16]) for a typing scheme.

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