***Salmonella* reference collection B (SARB): strains of 37 serovars of subspecies I**

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A reference collection of 72 strains representing 37 serovars of *Salmonella* subspecies I has been established for use in research on genetic and phenotypic variation in natural populations. Included are isolates of the host-adapted serovars *S. choleraesuis*, *S. dublin*, *S. gallinarum*, *S. paratyphi A*, *S. paratyphi B*, *S. paratyphi C*, *S. pullorum*, *S. sendai*, *S. typhi* and *S. typhisuis*, as well as strains of *S. enteritidis*, *S. typhimurium*, and other commonly recovered serovars with broad host ranges. The isolates were characterized by enzyme electrophoresis for allelic variation in 25 chromosomal genes and represent 71 distinctive multilocus genotypes (electrophoretic types or ETs). Genetic relationships among the ETs are indicated in an evolutionary tree constructed by the neighbour-joining method from a matrix of Nei's standard genetic distance.

Introduction

Among bacteria of the genus *Salmonella*, eight subgroups have been distinguished on the basis of hybridization of genomic DNA (Crosa *et al.*, 1973; Le Minor *et al.*, 1986), multilocus enzyme electrophoresis (MLEE) (Reeves *et al.*, 1989; Selander *et al.*, 1991), biotyping (Ewing, 1986; Le Minor *et al.*, 1986), and, most recently, nucleotide sequence analysis of chromosomal genes (Nelson *et al.*, 1991; Nelson & Selander, 1992). These groups are generally regarded as subspecies of a single species, designated as *S. enterica* (Le Minor & Popoff, 1987), but it has recently been proposed that the strains of group V are sufficiently differentiated from those of the other groups to warrant recognition as a distinct species, *S. bongori* (Reeves *et al.*, 1989).

Within the subspecies, strains are conventionally identified and classified according to the Kauffmann–White serotyping scheme, which is based on antigenic variation in the outer-membrane lipopolysaccharide (O) and phase 1 (H1) and phase 2 (H2) flagella (Le Minor 1984, 1988; Ewing, 1986). Of the 2200

serovars that have been distinguished, 99%, including all the major human pathogenic types, are members of subspecies I. Historically, serovars were designated by Latin binomials (e.g. *Salmonella typhimurium*), as if they were distinct species. This nomenclature is still widely used, although taxonomic schemes have recently been proposed in which the serovar names are used vernacularly (e.g. *S. enterica* serovar Typhimurium) (Ewing, 1986).

Serotyping is a convenient and epidemiologically useful method of categorizing isolates, but it does not provide a basis for estimating evolutionary genetic relatedness among strains. Studies of electrophoretically demonstrable allelic variation at multiple enzyme loci have recently demonstrated that isolates of the same serovar may be distantly related in chromosomal genotype, and that, conversely, strains of different serovars may be virtually identical in overall genetic character (Beltran *et al.*, 1988; Selander *et al.*, 1990*b*). For the phase I flagellin gene (*fliC*), comparative sequence analysis of multiple strains of *Salmonella* for which MLEE has provided an evolutionary framework has strongly implicated horizontal genetic transfer and substitutive recombination as a major process generating new serovars (Smith & Selander, 1990, 1991; Smith *et al.*, 1990). And there is evidence that gene transfer and recombination have also been extensively involved in the diversification of the *rfb* gene cluster mediating serotypic

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Abbreviations: ET, electrophoretic type; MLEE, multilocus enzyme electrophoresis; SARA, *Salmonella* reference collection A; SARB, *Salmonella* reference collection B.

Table 1. Properties of the 72 strains of the *Salmonella* reference collection B (SARB), representing 37 serovars of subspecies I

SARB no.	RKS no.	ET	Original number*	Source	Locality	Biotypet†	Date
<i>Salmonella agona</i> (1,4,12:f,g,s:--)							
1	1701	Ag 1	IVB 36/79	—	Peru	—	—
<i>Salmonella anatum</i> (3,10:e,h:1,6)							
2	2403	An 1	CDC B1487	Human	Washington	—	—
<i>Salmonella brandenburg</i> (1,4,12:l,v:e,n,z ₁₅)							
3	4231	Ba 2	DMS 2819	—	Scotland	10 i	1988
<i>Salmonella choleraesuis</i> (6,7:[c]:1,5)							
4	1280	Cs 1	NVSL 6321	Swine	Minnesota	—	1986
5	1239	Cs 6	IVB 651/79	—	Switzerland	—	—
6	3169	Cs 11	CDC 3327/54	—	Thailand	—	1954
7	4640	Cs 13	IP 6562/88	—	Australia	—	—
<i>Salmonella decatur</i> (6,7:c:1,5)§							
8	4647	Dt 1	IP 631 K	—	France	—	—
<i>Salmonella derby</i> (1,4,[5],12:f,g:[1,2])							
9	246	De 1	NVSL 4111	Avian	Oklahoma	—	1986
10	241	De 13	NVSL 5558	Swine	Minnesota	—	1986
11	243	De 31	NVSL 5283	Turkey	Pennsylvania	—	1986
<i>Salmonella dublin</i> (1,9,12,[Vi]:g,p:--)							
12	1518	Du 1	NVSL 5618	Cattle	Idaho	—	1986
13	4717	Du 3	IP 82/3144	Bovine	France	—	1982
14	1550	Du 2	IVB 3540/24	—	Thailand	—	—
<i>Salmonella duisburg</i> (1,4,12,27:d:e,n,z ₁₅)							
15	4239	Di 1	DMS 3618	—	Scotland	9 i	1988
<i>Salmonella enteritidis</i> (1,9,12:g,m:[1,7])							
16	53	En 1	CDC SSU7998	—	Rhode Island	—	—
17	761	En 2	IVB 176/82	—	Brazil	—	—
18	69	En 3	CDC SSU8074	—	Connecticut	—	—
19	1208	En 7	IVB 470/82	—	Switzerland	—	—
<i>Salmonella emek</i> (8,20:g,m,s:--)							
20	1216	Em 1	IVB 4793/3366	—	Israel	—	—
<i>Salmonella gallinarum</i> (1,9,12:--:--:--)							
21	2962	Ga 2	CDC 4801/72	Human	Connecticut	—	1972
<i>Salmonella haifa</i> (1,4,[5],12:z ₁₀ :1,2)							
22	4241	Ha 1	DMS 3005	—	Scotland	1 a	1988
<i>Salmonella heidelberg</i> (1,4,[5],12:r:1,2)							
23	539	He 1	NVSL 7039	Chicken	Pennsylvania	9 i	1987
24	1391	He 3	IVB 588/24	—	Thailand	1 a	—
<i>Salmonella indiana</i> (1,4,12:z:1,7)							
25	4250	Id 1	DMS 3702	—	Scotland	1 a	1988
<i>Salmonella infantis</i> (6,7:r:1,5)							
26	1490	In 1	CDC B3460	Human	North Carolina	—	—
27	1452	In 3	IVB 385/72	—	Senegal	—	—
<i>Salmonella miami</i> (1,9,12:a:1,5)							
28	2833	Mi 1	CDC 4648/53	Human	Georgia	—	1953
29	4381	Mi 5	IP 2/79	Human	French Guiana	—	1979
<i>Salmonella montevideo</i> (6,7:g,m,[p],s:[1,2,7])							
30	1762	Mo 1	CDC B2131	Human	Georgia	—	—
31	1740	Mo 6	CDC B2604	Human	Florida	—	—
<i>Salmonella muenchen</i> (6,8:d:1,2)							
32	3121	Mu 1	ATCC 8388	—	Laboratory strain	—	—
33	4288	Mu 2	IP 11/88	Human	France	—	1988
34	4300	Mu 3	IP 25/88	Human	France	—	1988
35	4272	Mu 4	CDC B1293	Human	North Carolina	—	—
<i>Salmonella newport</i> (6,8:e,h:1,2)							
36	2016	Np 8	CDC B3465	Human	North Carolina	—	—
37	1915	Np 11	INSP 15	Human	Mexico	—	—
38	1956	Np 15	NVSL 3882	Snake	Massachusetts	—	1987
<i>Salmonella panama</i> (1,9,12:l,v:1,5)							
39	1793	Pn 1	IVB Bendia	—	Italy	—	—
40	1776	Pn 2	CDC B1171	Human	North Carolina	—	—
41	1779	Pn 12	CDC B1433	Human	North Carolina	—	—
<i>Salmonella paratyphi</i> A (1,2,12:a:[1,5])							
42	4993	Pa 1	ATCC 9150	—	Laboratory strain	—	—

Table 1. (cont.)

SARB no.	RKS no.	ET	Original number*	Source	Locality	Biotype†	Date
<i>Salmonella paratyphi B</i> (including variety <i>java</i>) (1,4,[5],12:b:[1,2])							
43	3222	Pb 1	DMS 155/76	Human	France	3 gh	1976
44	3202	Pb 3	DMS 106/76	Food	Middle East	2 a	1976
45	3201	Pb 4	DMS 83/76	Human	France	13 i	1976
46	3274	Pb 5	DMS 2471	Water	United Kingdom	11 i	< 1965
47	3215	Pb 7	DMS 53/81	Human	Africa	1 g	1981
<i>Salmonella paratyphi C</i> (6,7[Vi]:c:1,5)							
48	4587	Pc 1	IP 33 K	—	France	—	—
49	4594	Pc 2	IP 2/88	Human	France	—	1988
50	4620	Pc 4	IP 4/77	Human	France	—	1977
<i>Salmonella pullorum</i> (1,9,12:--:--:--)#							
51	2266	Pu 3	IVB 978/87	—	Germany	—	—
52	2246	Pu 4	IVB Italian Standard	—	Germany	—	—
<i>Salmonella reading</i> (1,4,[5],12:e,h:1,5)							
53	4256	Re 1	DMS 3853	—	Scotland	2 j	1988
<i>Salmonella rubislaw</i> (11:r:e,n,x)							
54	4938	Ru 1	ATCC 10717	—	Laboratory strain	—	—
<i>Salmonella saintpaul</i> (1,4,[5],12:e,h:1,2)							
55	1690	Sp 3	CDC B2076	Human	Texas	—	—
56	1686	Sp 4	CDC B1400	Human	Florida	—	—
<i>Salmonella schwarzengrund</i> (1,4,12,27:d:1,7)							
57	4261	Sw 1	DMS 1253	—	Scotland	10 i	1988
<i>Salmonella sendai</i> (1,9,12:a:1,5)							
58	2866	Se 1	CDC 1035/74	Human	California	—	1974
<i>Salmonella senftenberg</i> (1,3,19:g,[s],t:--)							
59	2358	Sf 1	NVSL 6673	Chicken	Maryland	—	1987
<i>Salmonella stanley</i> (1,4,[5],12,27:d:1,2)							
60	4264	St 1	DMS 1112	—	Scotland	26 bei	1988
<i>Salmonella stanleyville</i> (1,4,[5],12,27:z ₄ ,z ₂₃ : [1,2])							
61	4267	Sv 2	DMS 3705	—	Scotland	9 i	1988
<i>Salmonella thompson</i> (6,7:k:1,5)							
62	1767	Th 1	CDC B2637	Human	Florida	—	—
<i>Salmonella typhi</i> (9,12,[Vi]:d:[Z ₆₆])							
63	3333	Tp 1	IP E. 88.374	—	Dakar	—	1988
64	3320	Tp 2	IP E. 88.353	—	Dakar	—	1988
<i>Salmonella typhimurium</i> (1,4,[5],12:i:1,2)							
65	284	Tm 1	INSP 24	Human	Mexico	1 f	—
66	203	Tm 7	NVSL 2816	Parrot	California	1 a	1987
67	837	Tm 12	IVB 1430	—	France	29 b	—
68	4535	Tm 23	USFW 318	Heron	Oregon	—	—
<i>Salmonella typhisuis</i> (6,7:c:1,5)							
69	3134	Ts 1	CDC 277/68	Swine	California	—	1968
70	3133	Ts 3	CDC 1426/67	Swine	Iowa	—	1967
<i>Salmonella wien</i> (1,4,12,27:b:1,w)							
71	4000	Wi 1	IP 5/88	Human	France	—	1988
72	3998	Wi 2	IP 3/88	Human	France	—	1988

* Abbreviations: ATCC, American Type Culture Collection; CDC, Centers for Disease Control (Atlanta, Georgia); DMS, University of Dundee Medical School (Ruth Barker collection) (Dundee, Scotland); INSP, Instituto Nacional de Salud Publica (Cuernavaca, Mexico); IP, Institut Pasteur (Paris, France); IVB, Institut für Veterinärmedizin des Bundesgesundheitsamtes (Berlin, FRG); NVSL, National Veterinary Services Laboratories (Ames, Iowa); USFW, United States Fish and Wildlife collection (Ruth Duncan collection) (Madison, Wisconsin).

† Biotypes determined by D. C. Old (personal communication). For explanation of biotype designations, see Duguid *et al.* (1975) and Barker *et al.* (1988).

‡ The serotype formula, indicating the O:H1:H2 antigenic factors, is shown in parentheses for each serovar. See Le Minor (1984) for detailed explanation.

§ Listed as a synonym of *S. choleraesuis* by Le Minor (1984).

|| Serotype 1,9,12:g,m,p:--. This strain has been serotyped as *S. dublin* but is distantly related to other strains of that serovar (see Selander *et al.*, 1992).

¶ Listed as *S. gallinarum-pullorum* by Le Minor (1984).

Listed as *S. gallinarum-pullorum* by Le Minor (1984) and as bioserovar Pullorum by Ewing (1986).

polymorphism in the somatic lipopolysaccharide of *Salmonella* (Brown *et al.*, 1992; Lee *et al.*, 1992; Wang *et al.*, 1992).

Because of increasing interest on the part of microbiologists and molecular biologists in analysing genetic and phenotypic variation in natural populations of

Table 2. Allele profiles at 24 polymorphic enzyme loci for ETs of the *Salmonella* reference collection B (SARB)

ET*	N†	Allele at indicated enzyme locus†:																							
		IDH	ACO	CAK	API	AP2	6PG	PGI	NSP	CAT	HEX	LG1	LG2	PLP	MDH	G6P	MIP	GDH	PGM	GLU	IPO	MPI	GOT	SKD	THD
Ag 1	114	3	3	3	3	10	3	3	5	3.5	3	3	3	3	3	3	3	3	2	3	3	3	3	0.5	3
An 1	33	2	3	3	3	8.2	2	3	5	3	3	3	2	3.5	7	3	3	3	3	3	3	3	3	6	3
Ba 2	1	3	4	3	5	14	3	3	5	2.5	3	3	3	3	3	3	4	3	4	3	3	3	3	4	3
Cs 1	131	4	3	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	3	3	3	6	3
Cs 6	1	3	3	3	3	8.2	3	3	3	3	5	3	3	3	3	2.9	2	3	2	3	3	3	3	1	3
Cs 11	3	3	2	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	0	3	3	6	3
Cs 13	2	3	3	3	3	11.5	2	3	5	3	3	3	3	3	3	2.9	3	3	3	3	3	3	3	1	3
Dt 1	10	3	2	3	5	8	3	3	5	2	3	3	0	3	3	3	3	3	2	3	3	5	3	1	3
De 1	77	3	2	3	3	3	3	4	5	3.5	3	3	2	4	3	3	2	3	2	3	3	3	3	6	3
De 13	220	2	3	3	3	11	5	3	5	1	3	3	2	3	3	3	3	3	3	3	3	3	3	1	3
De 31	20	3	2	3	3	8	3	3	5	3.5	3	3	3	3	3	3	3	3	3	3	3	3	3	6	3
Du 1	128	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3	3	1	3
Du 3	36	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3
Du 28	5	3	3	3	3	10	3	3	3	0.1	3	3	2	3	3	3	3	3	3	3	3	3	3	0.5	3
Di 1	2	2	3	3	3	14	3	3	5	2.5	3	3	2	3	3	3	3	3	3	3	3	3	3	1	3
En 1	357	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0.5	3
En 2	1	3	4	3	5	14	3	3	9	2	3	5	3	3	3	3	0.5	3	2	3	3	3	3	1	3
En 3	3	3	3	2	3	5	3	3	3	3	3	3	3	2.5	3	3	3	3	3	5	4	3	3	0.5	3
En 7	1	3	3	3	3	7	3	3	3	2.5	3	2	3	3	3	3	5	3	3	3	3	3	3	1	3
Em 1	1	3	4	3	3	7.5	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3
Ga 2	13	3	3	3	3	0	3	5	3.2	3	3	3	3	3	7	3	3	3	3	3	3	3	3	0.5	2
Ha 1	1	3	3	3	3	8.2	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	0.5	3
He 1	173	3	3	3	3	10	2	3	7	2.5	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3
He 3	3	2	3	3	3	10	2	3	7	2.5	3	3	3	3	3	3	3	3	3	3	3	3	3	6	3
Id 1	5	3	3	3	5	11	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3
In 1	109	3	3	3	3	11.5	3	3	3	4	3	3	3	3	3	3	3	3	2	3	3	3	3	6	2
In 3	1	3	3	3	5	11.8	3	3	5	3	3	4	2	3	3	3	3.5	3	2	3	3	6	3	1	3
Mi 1	18	2	3	3	5	14	3	3	5	2.5	3	6	3	3.5	3	3	2	3	2	3	3	3	3	1	3
Mi 5	3	3	3	3	5	14	3	3	5	2	3	4	3	3.5	3	3	2	3	2	3	3	5	3	1	3
Mo 1	38	5	3	3	3	14	3	3	5	3	6	4	2	4	3	5	2	3	2	3	3	2	3	1	3
Mo 6	3	5	3	3	5	14	3	3	5	2.5	3	4	2	4	3	3	2	3	2	3	3	2	3	1	3
Mu 1	46	3	5	3	3	14	2	3	7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0.5	3
Mu 2	19	3	5	3	5	14	2	3	5	4	3	3	3	3	3	3	3	2	2	3	3	3	3	1	3
Mu 3	4	2	3	3	5	14	3	3	3	3.5	3	3	3	3	3	3	3	3	3	3	3	3	3	0.5	3
Mu 4	2	3	3	3	3	7	3	3	5	3	3	2	3	3	2	3	3	3	3	3	3	3	3	0.5	3
Np 8	111	3	3	3	3	5	3	3	5	3	3	3	3	3	3	3	3	3	2	3	3	3	3	1	3
Np 11	228	3	3	3	3	7	3	3	5	3	3	3	3	3	6	3	3	3	3	3	3	3	3	0.5	3
Np 15	1	2	3	3	3	7	2	3	5	2.5	3	3	3	3	6	3	3	3	4	3	3	3	3	0.5	3

Pn 1	74	3	3	3	3	5	14	3	3	3	5	2-5	3	4	3	4	3	3	2	3	2	3	3	3	3	3	0-5	3
Pn 2	1	3	3	3	3	5	14	3	3	3	5	2-5	3	4	3	4	3	3	2	3	2	3	3	3	3	3	1	3
Pn 12	1	2	3	3	3	5	14	1	3	3	5	2-5	3	3	3	3	3	3	2	3	2	3	3	3	3	3	1	3
Pa 1	117	3	3	3	2	5	8	3	3	3	5	0	3	3	3	3-5	3	3	3	2	3	3-5	3	3	3	3	0-5	3
Pb 1	139	3	5	3	3	3	14	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Pb 3	46	2	5	3	3	3	0	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Pb 4	62	3	3	3	3	5	14	2	3	3	3	2	3	3	3	3	3	3	5	3	3	3	3	3	3	3	0-5	3
Pb 5	1	3	3	3	3	3	10	2	3	3	3	1-5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Pb 7	1	3	3	3	3	3	14	3	3	3	5	2	3	3	3	3	3	3	3	3	2	3	3	3	3	3	1	3
Pc 1	60	3	2	3	3	3	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	6	3
Pc 2	27	3	2	3	3	3	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3
Pc 4	4	3	3	3	3	5	14	3	3	3	5	2-5	3	4	2	4	3	3	3-5	3	2	3	3	3	3	3	1	3
Pu 3	21	3	2-5	3	0	5	3	5	3-2	3	3	3	3	3	4	0	3	3	3	3	3	3	3	3	3	2	0-5	2
Pu 4	9	3	2-5	3	0	0	3	3	5	2-9	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	2	0-5	2
Re 1	1	5	4	3	5	14	3	3	3	5	2	3	3	3	4	2	3	3	3-5	3	2	3	3	3	3	3	4	3
Ru 1	1	3	3	3	5	14	3	3	3	5	2-5	3	3	4	3	4	3	3	2	3	2	3	3	3	2	3	1	3
Sp 3	27	3	3	3	3	3	8-2	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Sp 4†	1	2	3	3	3	5	10-5	5-5	3	5	2-5	3	5	2	3	3	3	3	3	3	3	3	3	3	3	3	1	3
Sw 1	3	3	3	3	5	11	3	3	3	5	1-5	3	3	4	2	3	3	3	2	3	2	3	3	3	3	3	4	3
Se 1	1	3	3	3	2	0	5	3	3	5	0	3	3	3	3	3-5	3	3	2	3	2	3	3	3	3	3	0-5	3
Sf 1	67	3	5	3	3	3	3	3	3	5	3	3	3	3	2	4	3	3	3	3	3	3	3	3	3	3	1	3
St 1	1	2	3	3	3	3	14	2	3	3	2-5	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	1	3
Sv 2	1	2	3	4	3	3	9	3	3	5	2	3	3	3	4	5	3	3	6	3	3	3	3	3	3	2	1	3
Th 1	8	3	3	3	0	7	3	3	3	7	3-5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Tp 1	276	3	3	3	5	11-3	3	3	3	5	3	0	3	2	4	3	3	5	3	3	2	4	3	3	3	3	2	2
Tp 2	53	3	3	3	5	0	3	3	3	5	3	0	3	2	4	3	3	5	2-5	3	2	4	3	3	3	3	2	2
Tm 1	258	3	3	3	3	10	2	3	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Tm 7	2	3	3	3	3	10	2	3	3	7	4	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	0-5	3
Tm 12	27	3	3	3	3	10	2	3	3	0	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Tm 23	2	3	3	3	3	10	2	3	3	0	4	3-5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0-5	3
Ts 1	4	5	2	3	0	0	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	0	2	3	3	6	3
Ts 3	1	3	2	3	3	5	8	3	3	5	2	3	3	3	3	3	3	3	3	2	3	2	3	3	5	3	1	3
Wi 1	42	2	3	3	3	11-7	3	3	3	5	2-5	3	3	3	3	3	3	3	2	0	3	2	3	3	3	3	1	4
Wi 2	10	3	3	3	3	11-3	5	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3

* Serovar abbreviations: Ag, *S. agona*; An, *S. anatum*; Ba, *S. brandenburg*; Cs, *S. choleraesuis*; De, *S. derby*; Di, *S. disburg*; Dt, *S. decatur*; Du, *S. dublin*; Em, *S. enteritidis*; En, *S. enteritidis*; Ga, *S. gallinarum*; Ha, *S. haita*; He, *S. heidelberg*; Id, *S. indiana*; In, *S. infantis*; Mi, *S. miami*; Mo, *S. montevideo*; Mu, *S. muenchen*; Np, *S. newport*; Pa, *S. paratyphi A*; Pb, *S. paratyphi B*; Pc, *S. paratyphi C*; Pn, *S. panama*; Pu, *S. pullorum*; Re, *S. reading*; Ru, *S. rubislaw*; Se, *S. sendai*; Sf, *S. senftenberg*; Sp, *S. saintpaul*; St, *S. stanley*; Sv, *S. stanleyville*; Sw, *S. schwarzengrund*; Th, *S. thompson*; Tm, *S. typhimurium*; Tp, *S. typhi*; Ts, *S. typhus*; Wi, *S. wien*.

† Number of isolates examined.

‡ Enzyme locus symbols: IDH, isocitrate dehydrogenase; ACO, aconitase; CAK, carbamate kinase; API, acid phosphatase-1; AP2, acid phosphatase-2; 6PG, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; NSP, nucleoside phosphorylase; CAT, catalase; HEX, hexokinase; LG1, leucylglycyl-glycine peptidase-1; LG2, leucylglycyl-glycine peptidase-2; PLP, phenylalanyl-leucine peptidase; MDH, malate dehydrogenase; G6P, glucose-6-phosphate dehydrogenase; MIP, mannitol-1-phosphate dehydrogenase; GDH, glucose dehydrogenase; PGM, phosphoglucomutase; GLU, glutamate dehydrogenase; IPO, indophenol oxidase; MPI, mannose phosphate isomerase; GOT, glutamic oxaloacetic transaminase; SKD, shikimate dehydrogenase; THD, threonine dehydrogenase. One additional enzyme locus – ADK, adenylate kinase – (not shown) was monomorphic. For each enzyme locus, alleles are numbered in order of decreasing electrophoretic mobility of their corresponding electromorphs; 0 indicates a null allele.

§ Alleles of CAT and SKD were scored as 1 and 5, respectively, by Selander *et al.* (1992).

|| PGI was scored as a 4 allele by Beltran *et al.* (1991).

¶ CAT was scored as a 3 allele by Beltran *et al.* (1991).

Salmonella, we have prepared several research reference collections of strains. Beltran *et al.* (1991) designated a set of 72 strains of the *S. typhimurium* complex (including representatives of the serovars *S. typhimurium*, *S. saintpaul*, *S. heidelberg*, *S. paratyphi B* and *S. muenchen*) as the *Salmonella* reference collection A (SARA). We here report the availability of the *Salmonella* reference collection B (SARB), which consists of 72 strains representing 37 serovars of subspecies I, including the medically important human pathogenic forms.

Methods

Bacterial strains. In selecting the SARB strains (Table 1) from a collection of several thousand isolates that have been characterized in our laboratory by MLEE, we attempted to maximize representation of the genomic diversity within subspecies I. The strains are of 37 serovars; and for each of 19 serovars for which preliminary MLEE analyses had identified multiple electrophoretic types (ETs), we selected one isolate of the most frequently recovered ET and single isolates of one or more other ETs in order to reflect the total span of multilocus enzyme diversity among strains of the serovar. A total of 12 strains in SARB are also included in SARA.

MLEE. Electrophoretically demonstrable allelic variation in 25 chromosomal genes encoding metabolic enzymes was analysed by the methods described by Selander *et al.* (1986). Each isolate was grown overnight in 150 ml brain heart infusion broth (Difco) at 37 °C on an orbital shaker (250 r.p.m.) and harvested by centrifugation at 6000 *g* for 10 min at 4 °C. Pelleted cells were resuspended in 2 ml 50 mM-EDTA (pH 7.5); the bacteria were sonicated with a Sonifier Cell Disruptor (Branson Sonic Power Co.) with a microtip for 30 s at 50% pulse, with ice-water cooling; and a lysate was obtained by centrifugation at 20000 *g* for 20 min at 4 °C.

Lysates were electrophoresed on starch gels and selectively stained for 25 enzymes. Electromorph variants of an enzyme, numbered in order of decreasing anodal mobility, were equated with alleles at the corresponding structural gene locus. An absence of enzyme activity was attributed to a null allele (Selander *et al.*, 1986), and all such null scores were verified by regrowing the strains and electrophoresing freshly prepared lysates. The enzymes studied and the allele profiles (multilocus enzyme genotypes) of the 72 SARB strains are shown in Table 2.

Statistical analysis. Statistical analyses were carried out by computer programs written by T. S. Whittam, Institute of Molecular Evolutionary Genetics. Estimates of genetic relationships among the ETs are indicated in the evolutionary tree in Fig. 1, which was generated by the neighbour-joining method (Saitou & Nei, 1987) from a pairwise

Fig. 1. Evolutionary tree showing relationships among the 71 ETs represented by the 72 SARB strains. See text for explanation. Abbreviations of serovars: Ag, *S. agona*; An, *S. anatum*; Ba, *S. brandenburg*; Cs, *S. choleraesuis*; De, *S. derby*; Di, *S. disburg*; Dt, *S. decatur*; Du, *S. dublin*; Em, *S. emek*; En, *S. enteritidis*; Ga, *S. gallinarum*; Ha, *S. haifa*; He, *S. heidelberg*; Id, *S. indiana*; In, *S. infantis*; Mi, *S. miami*; Mo, *S. montevideo*; Mu, *S. muenchen*; Np, *S. newport*; Pa, *S. paratyphi A*; Pb, *S. paratyphi B*; Pc, *S. paratyphi C*; Pn, *S. panama*; Pu, *S. pullorum*; Re, *S. reading*; Ru, *S. rubislaw*; Se, *S. sendai*; Sf, *S. senftenberg*; Sp, *S. saintpaul*; St, *S. stanley*; Sv, *S. stanleyville*; Sw, *S. schwarzengrund*; Th, *S. thompson*; Tm, *S. typhimurium*; Tp, *S. typhi*; Ts, *S. typhisuis*; and Wi, *S. wien*.

matrix of Nei's standard genetic distance (Nei, 1987) for the 25 enzyme loci assayed. Distance was calculated as $d_{ij} = -\ln(1-D)$, where D equals the proportion of loci with different alleles between the i th and j th ETs. Under the assumptions that any allelic difference in electrophoretic mobility results from at least one codon difference at the nucleotide level and that codon changes occur independently, d estimates the mean number of electrophoretically detectable codon differences per genetic locus (Nei, 1987; Herzer *et al.*, 1990).

As drawn in Fig. 1, the tree is unrooted, but clustering of the SARB ETs with ETs of the other seven *Salmonella* subspecies as outgroups (data and analysis not shown) placed the root on the line leading to the two ETs of *S. typhi* (Tp 1 and Tp 2), in cluster E. For convenience of reference, seven divisions of the tree are designated by letter.

Results and Discussion

MLEE analysis of the 72 SARB isolates (Table 1) identified 71 distinctive ETs (Table 2). Isolate RKS 4241 (SARB 22), representing *S. haifa* (ET Ha 1), was indistinguishable in allele profile from isolate RKS 1690 (SARB 55), representing Sp 3 of *S. saintpaul*.

Previous studies have shown that the population structure of *Salmonella* is clonal, with each serovar being represented by one or a few predominant clones of widespread if not global distribution (Beltran *et al.*, 1988; Reeves *et al.*, 1989; Selander *et al.*, 1990a,b, 1992). Of the 19 serovars that are represented in SARB by two or more ETs, eight (*S. heidelberg*, *S. miami*, *S. montevideo*, *S. panama*, *S. pullorum*, *S. typhi*, *S. typhimurium* and *S. wien*) are apparently monophyletic; the ETs of each of these serovars are genotypically similar and presumably have been derived through mutational divergence from a recent common ancestor. But the other 11 serovars (*S. choleraesuis*, *S. derby*, *S. dublin*, *S. enteritidis*, *S. infantis*, *S. muenchen*, *S. newport*, *S. paratyphi B*, *S. paratyphi C*, *S. saintpaul* and *S. typhisuis*) are each polyphyletic, as previously noted (Beltran *et al.*, 1988, 1991; Selander *et al.*, 1990a,b, 1992; Li *et al.*, 1993), with, in most cases, ETs occurring in two or more divisions of the evolutionary tree (Fig. 1).

Genetic variation and evolutionary relationship within and among several of the serovars included in SARB have previously been studied in detail. In an analysis of multilocus enzyme genotypes and sequence variation in the *fliC* gene, the flagellar hook-associated protein 1 gene (*flgK*), and the 6-phosphogluconate dehydrogenase gene (*gnd*), Li *et al.* (1993) concluded that strains of the non-motile avian-adapted biovars *S. gallinarum* and *S. pullorum* shared a most recent common ancestor and are phylogenetically related to the common clonal lineage, En 1, of *S. enteritidis*. In the present analysis (Fig. 1), Ga 2 clusters with Pu 3 and Pu 4 and occurs with En 1, En 3 and En 7 in division A. But *S. enteritidis* is a polyphyletic serovar (Beltran *et al.*, 1988), and En 2 (in division F) is distantly related to other ETs of that serovar, except in the sequence of its *fliC* gene, which was

apparently acquired by horizontal transfer (Li *et al.*, 1993).

A close relationship between ETs Du 1 and Du 3 of *S. dublin* and En 1 and similar ETs of *S. enteritidis*, as shown by Selander *et al.* (1992), is indicated in Fig. 1 (division A). Du 2 also occurs in division A but clusters with the main group of *S. typhimurium* and *S. paratyphi B* ETs. Similarly, most of the ETs of *S. paratyphi B* are allied with those of *S. typhimurium* (division A), but Pb 7 (at the bottom of the tree) is only distantly related to the other ETs of *S. paratyphi B* (Selander *et al.*, 1990a; Beltran *et al.*, 1991).

The three ETs of *S. derby* included in SARB differ from each other, on average, at nine enzyme loci and occur in three divisions (A, B and E) of the evolutionary tree (Fig. 1), reflecting multiple evolutionary origins (Beltran *et al.*, 1988).

SARB includes isolates of several medically important serovars for which MLEE analysis has identified infrequently recovered ETs that are distantly related to the common and widespread ET of the serovar. *S. infantis* In 3 (division F), which is known from a single strain isolated in Senegal, differs from the globally distributed In 1 (division C) at 10 enzyme loci; *S. paratyphi C* Pc 4 (division F) differs from Pc 1 and Pc 2 (division B) at 11 and 12 loci, respectively (Selander *et al.*, 1990b); and *S. choleraesuis* Cs 1 and Cs 11 are closely related to one another and to *S. paratyphi C* Pc 1 and Pc 2 (division B), but Cs 6 (division C) and Cs 13 (division A) represent phylogenetically distinct lineages.

SARB also includes several examples in which different serovars are represented by closely related ETs. For example, *S. haifa* Ha 1 and *S. saintpaul* Sp 3, representing serovars that differ in expression of phase 1 flagellar antigenic factors (Table 1), were indistinguishable in multilocus enzyme genotype (Table 2).

Both clones of *S. typhi* (Tp 1 and Tp 2) that were identified by Selander *et al.* (1990b) are represented in SARB; they differ at two enzyme loci and cluster with *S. derby* De 1 and *S. senftenberg* Sf 1 in division E.

Recent comparative studies of nucleotide sequence variation in *fliC*, the *rfb* gene cluster, and several metabolic and other enzyme genes among diverse strains of *Salmonella* have suggested that recombination of horizontally transferred DNA segments has been an especially important evolutionary factor generating allelic and multilocus genotypic diversity in genes involved in serotype determination, presumably because of an adaptive advantage of high levels of polymorphism in cell-surface structures exposed to the environment (Selander *et al.*, 1991; Nelson & Selander, 1992). This hypothesis is currently being tested by sequence analysis of additional genes encoding proteins of a variety of functional types. For present purposes, it has been

sufficient to emphasize the fact that, because of recombination (and, perhaps, convergent evolution in antigenic phenotype), identity or similarity in serotype among strains of the salmonellae does not necessarily reflect close phylogenetic relationship. Estimates of evolutionary genetic relationships among strains must be based on assessment of allelic variation in large numbers of diverse chromosomal genes.

A set of SARB has been deposited in the *Salmonella* Genetic Stock Centre (contact Dr K. E. Sanderson, Department of Biological Sciences, University of Calgary, 200 University Drive NW, Calgary, Alberta, Canada T2N 1N4).

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