The *Xba*I-*Bln*I-*Ceu*I Genomic Cleavage Map of *Salmonella paratyphi* B

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The genomic cleavage map of *Salmonella paratyphi* B was determined through digestion with endonucleases and separation of the fragments by pulsed-field gel electrophoresis. The chromosome has 19 *Xba*I sites, 10 *Bln*I sites, and 7 *Ceu*I sites. The fragments were arranged in order through excision of fragments from the gel, redigestion with a second enzyme, end labelling with ^{32}P , and reelectrophoresis. *Tn10* transposons inserted in 61 different genes of *S. typhimurium* LT2 were transduced by use of bacteriophage P22 into *S. paratyphi* B. The locations of *Tn10* insertions on the chromosome of *S. paratyphi* B were determined by use of *Xba*I and *Bln*I sites in *Tn10*, revealing the positions of genes with *Tn10* insertions in *S. paratyphi* B. All seven *Ceu*I sites (in *rri* genes for 23S rRNA) and most of the *Xba*I and *Bln*I sites in *rrn* genes for Glt-tRNA are conserved, but only about half of the *Xba*I and *Bln*I sites outside *rrn* genes are conserved. Gene order is identical in the 68 genes that we could compare between *S. paratyphi* B and *S. typhimurium* LT2, and the lengths of intervals between the genes are often the same, but there are several instances of differences in interval lengths, indicating that insertions or deletions of DNA have occurred during the evolutionary divergence of these bacteria.

The linkage maps of *Salmonella typhimurium* (39) and *Escherichia coli* K-12 (2) and the physical maps of the genomes, based on either genomic cleavage maps or nucleotide sequences (21–23, 25, 30, 35, 36, 43), reveal that the overall chromosome structure and the gene order are strongly conserved between the two genera (37). However, the nucleotide sequences of homologous genes in the two genera have diverged significantly; for example, divergence in proline permease (*putP*) sequences between *S. typhimurium* and *E. coli* is 16.3%, whereas different serovars of the same genus differ by only 0.5 and 2.4% for members of the genera *Salmonella* and *Escherichia*, respectively (28). High (usually greater than 80%) levels of DNA-DNA reassociation and low ΔT_m values for DNAs within the genera but much lower levels of DNA reassociation and higher ΔT_m values between the genera (9) also indicate substantial nucleotide sequence divergence between the genera. (ΔT_m is the thermal elution midpoint, i.e., the temperature at which 50% of the DNA bound to hydroxyapatite is eluted.) Thus, it has been widely believed that nucleotide sequence changes rather than chromosomal rearrangements explain the evolution of these genera. However, both large and small genomic rearrangements have occurred in the evolution of different genera of enteric bacteria. An example of a large rearrangement is the inversion of about 315 kb (22) in the terminal region of the chromosomes (8, 38) of *S. typhimurium* and *E. coli*. In addition, small insertions or deletions distinguish the linkage maps of *E. coli* and *S. typhimurium* (15, 33, 34). For example, *E. coli* ferments lactose, while *S. typhimurium* is Lac[−]; loops of DNA which carry several genes, including *lac*, are present in *E. coli* but missing from *S. typhimurium* (7, 16). More data are needed to clarify the relative roles of sequence divergence in homologous genes and chromosomal rearrangements in the evolution of different genera. The objective of this study was to use methods of

physical genome analysis to determine whether the chromosomal rearrangements of the types found between different genera are also found in different strains which are members of the same genus, *Salmonella*.

We have studied *S. paratyphi* B because it is closely related to but distinct from *S. typhimurium* and because it includes serious human pathogens. *Salmonellae* of serotype 1,4,(5),12:b:(1,2) are generally known as *S. paratyphi* B; the name *S. schottmuelleri* has been used (18), but most workers, including clinicians, use the name *S. paratyphi* B. We continue to use species designations such as *S. paratyphi* B rather than designations such as *S. enterica* serovar *paratyphi* B because of their historical use, although we recognize that members of this genus, especially those belonging to the same subgenus, are members of a single species, as defined by DNA-DNA reassociation (9, 19, 31). *S. paratyphi* B was initially described as the causal agent of typhoidlike enteric fever in humans. Later, it was recognized that some strains of this serotype do not cause enteric fever but instead produce an acute but mild gastroenteritis. Isolates which cause enteric fevers usually do not ferment *d*-tartrate but form an extracellular slime layer, whereas isolates which cause gastroenteritis are usually able to utilize *d*-tartrate but are deficient in the slime layer. Kauffmann (14) concluded that there are two species of identical serotype, and he proposed the name *S. java* for the *d*-tartrate-positive form and the name *S. paratyphi* B for the *d*-tartrate-negative form. Le Minor et al. (20) proposed that the name *S. java* be dropped and that these strains be considered a biovar of *S. paratyphi* B; we have adopted this usage, as have others (3, 41).

We report here the genomic cleavage map of *S. paratyphi* B IP87/87 for the enzymes I-*Ceu*I, *Xba*I, and *Bln*I (the designation “I” in enzyme I-*Ceu*I indicates that the enzyme is encoded by a class I intron; I-*Ceu*I will be called *Ceu*I in this report). Strain IP87/87 is of the tartrate-positive type and is electrophoretic type Pb3; it would have been called *S. java* by Kauffmann (14). We transduced *Tn10* transposons located in genes of *S. typhimurium* LT2 into *S. paratyphi* B and established the locations of these genes by locating the new *Xba*I and *Bln*I sites in *Tn10*. Gene order is identical in the 68 genes

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FIG. 1. Digestion of genomic DNA of *S. paratyphi* B IP87/87 with *CeuI*, *XbaI*, and *BlnI* and separation by PFGE. Panels A, B, and D were stained with ethidium bromide; panel C is an autoradiograph of the gel from panel B in which the DNA fragments were end labelled with ^{32}P prior to electrophoresis. Panels B, C, and D were all from the same gel; panel A is from an independent gel. The fragments are designated by uppercase letters and arranged alphabetically on the chromosome (see Fig. 2). In panel D, a concatamer (Con) of λ was digested with *HindIII*. The sizes of the fragments, in kilobases, were obtained from a comparison with λ concatamers or from the sums of the fragments obtained by excising fragments and redigesting them with another enzyme. Smaller fragments not visible with ethidium bromide (B) are visible after end labelling (C). In a few cases, the sizes of fragments have been rounded to the nearest 10.

that we tested for *S. paratyphi* B and *S. typhimurium* LT2, but only about half of the *XbaI* and *BlnI* sites outside *rrn* genes are conserved. We also found evidence of insertions or deletions of genetic material in *S. paratyphi* B relative to *S. typhimurium* LT2.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, plasmids, cultivation, and transduction conditions. The wild-type strain analyzed in detail is *S. paratyphi* B IP87/87; this strain was provided by Institut Pasteur, Paris, France, to the laboratory of R. K. Selander; there it was given the designation RKS3614, analyzed by multilocus enzyme electrophoresis, placed in electrophoretic type Pb3, and placed in the salmonella reference A (SARA) set of strains as SARA52 (4); this set is available from the Salmonella Genetic Stock Centre (SGSC). Other strains of *S. paratyphi* B that belong to the SARA set are also described here.

The strains of *S. typhimurium* LT2 and the plasmids used in this study were obtained from numerous sources and are maintained at the SGSC. Strains with insertions of transposon *Tn10* at different points around the linkage map are part of a "kit" of several hundred strains of *S. typhimurium* LT2 maintained at the SGSC; the kit was reported earlier (39) and has expanded substantially since that time. Transposons were transduced from *S. typhimurium* into *S. paratyphi* B by use of bacteriophage P22 HT105/int with selection for tetracycline resistance to detect transductants; see Table 2 for the *S.*

paratyphi B transductants. In all cases in which *Tn10* of *S. typhimurium* was inserted into a gene with a readily testable phenotype (e.g., insertion into a gene resulting in auxotrophy), the transductant of *S. paratyphi* B was confirmed to have the same phenotype. Practically all transductants tested had the expected phenotype, and only these were used in subsequent analyses. For all the *S. paratyphi* B transductants tested here, the expected phenotype was observed, but the tests were not done for some strains. These strains are available upon request.

Luria-Bertani medium (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, 3.5 ml of 1 M NaOH) was used for the cultivation of all strains; solid medium also contained 1.5% agar. Tetracycline was used at 20 $\mu\text{g}/\text{ml}$. Strains were maintained in 15% glycerol at -70°C , and a single colony was isolated prior to use.

Transduction was done with bacteriophage P22 HT105/int (40), propagated in broth on the donor strain, by spreading the phage preparation on cells of the recipient strain on a plate containing Luria-Bertani agar plus tetracycline.

Enzymes and chemicals. Endonucleases were from New England Biolabs (*XbaI* and *CeuI*), Takara Biochemicals (*BlnI*), Pharmacia, and Boehringer Mannheim Biochemicals. [^{32}P]dCTP was from New England Nuclear. Most other chemicals, including agarose, were from Sigma Chemical Co.

Techniques used in PFGE. Preparation and digestion of high-molecular-weight genomic DNA, digestion of DNA in agarose blocks, separation of DNA by pulsed-field gel electro-

TABLE 1. *CeuI* fragments from the genome of *S. paratyphi* B IP87/87

<i>CeuI</i> fragment ^a	Size		<i>CeuI</i> site at the counterclockwise end		Gene at <i>CeuI</i> site ^f	Fragment recut ^g by:		
	kb ^b	CS ^c	No. ^d	Location ^e		<i>XbaI</i>	<i>BlnI</i>	
				kb				CS
A	2,375	51.0	1	352	7.6	<i>rrlH</i>	270 ('B), 750 (C), 52 (D), 160 (E), 80 (F), 390 (G), 108 (H), 220 (I), 106 (J), 238 (K')	855 ('A), 72 (B), 650 (C), 800 (D')
B	695	14.9	2	2,725	58.5	<i>rrlG</i>	2.3 ('K), 48 (L), 645 (M')	2.2 ('D), 130 (E), 415 (F), 150 (G')
C	527	11.3	3	3,420	73.4	<i>rrlD</i>	2.3 ('M), 20 and 6.4 (N, O), 310 (P), 110 and 75 (Q, R), 2.3 (S')	2.2 ('G), 520 (H), 2.2 (I')
D	92	2.0	4	3,947	84.8	<i>rrlC</i>	92 ('S')	92 ('I')
E	145	3.1	5	4,039	86.7	<i>rrlA</i>	145 ('S')	145 ('I')
F	44	0.9	6	4,185	89.9	<i>rrlB</i>	42 ('S), 2.3 (A')	42 ('I), 2.2 (J')
G	780	16.8	7	4,229	90.8	<i>rrlE</i>	778 ('A), 2.3 (B')	290 ('J), 490 (A')

^a The fragments were determined from data of the type shown in Fig. 1 and designated by letter in order from the *thr* gene.

^b Based on PFGE of the fragment in comparison with the lambda concatamer or on the sum of the fragments obtained by digestion with a second enzyme (see the text).

^c The chromosome is divided into 100 units called centisomes (CS) (35, 36). Each CS in *S. paratyphi* B equals about 46.5 kb.

^d The sites are numbered in order around the chromosome.

^e The positions of enzyme cleavage sites around the chromosome are measured from the position of *thr* (as defined by the positions of the *XbaI* and *BlnI* sites in Tn10 inserted in *thr*) (see Table 2).

^f The gene associated with the cleavage site can be inferred in certain cases, primarily by analogy with the positions of *rrn* genes in *E. coli* (6, 21) and *S. typhimurium* (21, 22) (see the text).

^g The fragments were separated by PFGE, excised from agarose blocks, redigested with the indicated enzyme, end labelled with ³²P, electrophoresed, and autoradiographed. The sizes were correlated with digests of whole genomic DNA. Fragments marked "X" are inferred to be from the counterclockwise end of the fragment, those marked "X'" are inferred to be from the clockwise end of the fragment, and those marked "X'" are inferred to be from the middle of the fragment. For example, *XbaI* digestion of *CeuI*-E gives rise to a 145-kb fragment, *XbaI*-S'.

phoresis (PFGE), and Southern blotting were all carried out by methods reported earlier (22, 24).

Isolation, redigestion, and end labelling of DNA fragments. Genomic DNA fragments from endonuclease digestions were excised from agarose gels, redigested with a second endonuclease, end labelled with ³²P, reelectrophoresed, and autoradiographed according to methods described by Liu et al. (22, 24).

RESULTS

***CeuI* cleavage map.** The endonuclease *CeuI* recognizes and cleaves at a site 19 to 26 bp long in the 23S rRNA gene of *Chlamydomonas eugametos* (10, 26), and it cleaves the same sites in the seven *rrl* genes of *E. coli* and *S. typhimurium* (24). As expected, digestion of *S. paratyphi* B genomic DNA yielded seven fragments similar in size to those from other enteric bacteria (Fig. 1A). We isolated the *CeuI* fragments from the gel, redigested them with a second enzyme (either *XbaI* or *BlnI*), end labelled them, and reelectrophoresed them; the fragment sizes are listed in Table 1. The *CeuI* sites were numbered from 1 to 7, and the seven *CeuI* fragments were arranged in alphabetical order on the chromosome (Table 1 and Fig. 2).

***XbaI* cleavage map.** Digestion with *XbaI* produced 19 fragments, 18 of which were 20 kb or larger in size and were recognized following ethidium bromide staining (Fig. 1B), while 1, which was 6.4 kb in size, was recognized only after end labelling (Fig. 1C). (Fragments of similar lengths produced doublets, e.g., *XbaI*-Q and *XbaI*-H at 110 and 108 kb; these were distinguished by use of Tn10 insertions in one of the fragments; see below). The order of the *XbaI* fragments on the chromosome was determined as follows. First, Tn10 insertions at known locations on the chromosome of *S. typhimurium* were transduced with P22 into *S. paratyphi* B; *XbaI* digestion of genomic DNA from the transductants showed the loss of one

normal fragment and the formation of two smaller fragments. In most cases, the Tn10 insertion produced a specific phenotype (e.g., threonine requirement in the *S. paratyphi* B strain with the *thr*-Tn10 insertion); thus, Tn10 was known to have entered a homologous site in the transductants, and the physical location of the gene (*thr*, in this case) was determined. An analysis of 61 strains of *S. paratyphi* B with Tn10 insertions is shown in Table 2. Second, the *XbaI* fragments were excised from the gel and redigested with *BlnI* and *CeuI* (Table 3). With these data, the *XbaI* fragments were placed in alphabetical order (Table 3 and Fig. 2). The order of the small fragments *XbaI*-N (20 kb) and *XbaI*-O (6.4 kb) might be reversed.

***BlnI* cleavage map.** *BlnI* digestion yielded 10 fragments, ranging in size from 72 to 1,350 kb (Fig. 1B); fragments as small as 1 kb could have been detected but were not seen when end labelling was done (Fig. 1C; unpublished data). These 10 fragments were arranged in alphabetical order on the chromosome by the same methods as those used for *XbaI* (Table 4 and Fig. 2).

Evidence for an insertion in *S. typhimurium* LT2. *S. typhimurium* LT2 has sialidase (neuraminidase) activity because of the *nanH* gene; this gene and the enzyme are not present in most *Salmonella* strains (13). Using 4-methylumbelliferone as a sialidase substrate (13), we confirmed that only LT2 among the strains in the SARA set has sialidase activity (data not shown). *nanH*::Tn10 is at 1,140 kb in *S. typhimurium*, very close to a *BlnI* site and in a region of about 45 kb between *pncB* and *pyrD*; however, in *S. paratyphi* B, the genes *pncB*, *pepN*, and *pyrD* are all very close together, and the *nanH* gene and the *BlnI* site are missing (Fig. 3). These data indicate a DNA insertion in LT2 and its absence in *S. paratyphi* B; we confirmed this conclusion by using transduction. A P22 phage propagated on an *S. typhimurium* LT2 strain with a *pncB*::Tn10 insertion was used to transduce *S. typhimurium* SA2676 *pyrD*121 to tetracycline resistance. Of 120 transductants, none were *PyrD*⁺, indicating no joint transduction of *pncB* and *pyrD*,

FIG. 2. Genomic cleavage map of *S. paratyphi* B IP87/87. Cleavage sites for *XbaI*, *CeuI*, and *BlnI* are in kilobases and in centisomes (CS). The chromosome is about 4,650 kb in size. The fragments and the cleavage sites are in order, starting with fragment A; for example, site *CeuI*-1 and site *CeuI*-2 are on either side of fragment A (*CeuI*-A). Genes illustrated on the outer circle were mapped by use of Tn10 insertions in the *XbaI* and *BlnI* sites of Tn10 (see Table 2). The seven *rrn* genes were mapped by redigestion of *CeuI* fragments with *XbaI* or *BlnI* (Table 1). Arrows indicate the direction of transcription established for the homologous *rrn* genes of *E. coli*. Solid bars in the *rrn* gene diagrams indicate the gene order, which is *rrs* (16S rRNA)-*tRNA-rrl* (23S rRNA)-*rrf* (5S rRNA), in order of transcription, as shown by the arrows (6, 29). The tRNA genes next to the arrows are as in *E. coli*, except that *gltT* is in *rrnD* at 3,420 kb rather than in *rrnB* at 4,185 kb (17).

as expected, since the genes are 45 kb apart (Fig. 3), about the size of P22. However, a P22 phage propagated on *S. paratyphi* B IP87/87 with the same *pncB*::Tn10 insertion yielded 8 PyrD⁺ strains of 37 transductants tested, indicating about 20% joint transduction. According to the formula of Wu (44) as used by

Sanderson and Roth (39), this result indicates a distance of 14 kb between the genes, rather than only the few kilobases indicated by physical analysis (Table 2 and Fig. 3); however, in these interspecies crosses, joint transduction may be reduced and apparent distance may be increased. These data support

TABLE 2. Strains of *S. paratyphi* B IP87/87 and determination of the locations of their insertions on the genome by digestion with *Xba*I and *Bln*I

Allele no. of Tn10 insertion ^a	Strain of <i>S. paratyphi</i> B IP87/87 ^b	<i>Xba</i> I analysis ^c			<i>Bln</i> I analysis ^d			Location (kb) ^e
		Normal	A (counterclockwise)	B (clockwise)	Normal	A (counterclockwise)	B (clockwise)	
<i>thr-557</i>	SA4570 ^f	A (780)	435	355	A (1,348)	140	1,208	0
<i>pyrA234</i>	SA4613 ^f	A (780)	510	280	A (1,348)	215	1,133	75
<i>leu-1151</i>	SA4566 ^f	A (780)	560	220	A (1,348)	265	1,085	125
<i>pan-540</i>	SA4568 ^f	A (780)	645	125	A (1,348)	350	1,000	210
<i>proA1656</i>	SA4571 ^f	B (272)	35	245	A (1,348)	520	830	380
<i>purE884</i>	SA4564 ^f	B (272)	255	25	A (1,348)	740	610	600
<i>ahp-11</i>	SA4621 ^g	C (750)	50	710	A (1,348)	805	545	667
<i>nadA213</i>	SA4569 ^f	C (750)	210	560	A (1,348)	965	385	827
<i>bio-102</i>	SA4567 ^f	C (750)	245	515	A (1,348)	1,000	350	862
<i>aroA554</i>	SA4647 ^f	C (750)	405	355	A (1,348)	1,160	190	1,022
<i>aspC409</i>	SA4611 ^f	C (750)	430	330	A (1,348)	1,185	165	1,047
<i>pepN88</i>	SA4624 ^g	C (750)	445	315	A (1,348)	1,200	148	1,063
<i>pncB150</i>	SA4614 ^g	C (750)	447	313	A (1,348)	1,200	146	1,063
<i>pyrD2266</i>	SA4565 ^f	C (750)	448	312	A (1,348)	1,200	145	1,063
<i>putA</i>	SA4784 ^g	C (750)	515	245	A (1,348)	— ^h	—	1,132
<i>pyrC691</i>	SA4627 ^f	C (750)	550	210	A (1,348)	1,308	40	1,167
<i>flgL</i>	SA4600 ^f	C (750)	565	195	A (1,348)	1,328	20	1,182
<i>ptsG4152</i>	SA4629 ^g	C (750)	604	156	B (72)	3	70	1,211
<i>tpxB16</i>	SA4625 ^g	E (160)	86	84	B (72)	215	435	1,505
<i>oxrA2</i>	SA4620 ^g	G (390)	50	350	B (72)	450	215	1,709
<i>cysB3305</i>	SA4602 ^f	G (390)	100	300	B (72)	NC ⁱ	—	1,759
<i>trp-2451</i>	SA4572 ^f	G (390)	120	280	B (72)	NC	—	1,779
<i>dadB5</i>	SA4631 ^f	G (390)	200	200	B (72)	NC	—	1,859
<i>dadB7</i>	SA4630 ^f	G (390)	200	200	B (72)	NC	—	1,859
<i>zea-1437</i>	SA4626 ^g	G (390)	317	83	D (800)	48	752	1,976
<i>cheY</i>	SA4617 ^g	G (390)	327	73	D (800)	58	742	1,986
<i>zea-4</i>	SA4618 ^g	G (390)	341	59	D (800)	72	728	2,000
<i>fluC</i>	SA4597 ^f	G (390)	355	45	D (800)	85	715	2,014
<i>cob-2</i>	SA4573 ^f	H (108)	35	82	D (800)	155	645	2,084
<i>cob-4</i>	SA4574 ^f	H (108)	35	82	D (800)	150	650	2,084
<i>cob-10</i>	SA4575 ^f	H (108)	35	82	D (800)	150	650	2,084
<i>hisC8667</i>	SA4576 ^f	H (108)	92	25	D (800)	210	590	2,142
<i>hisE9446</i>	SA4577 ^f	H (108)	92	25	D (800)	210	590	2,142
<i>ompC396</i>	SA4623 ^g	I (220)	185	45	D (800)	410	390	2,342
<i>ack-408</i>	SA4596 ^g	J (106)	65	50	D (800)	510	290	2,442
<i>purF1714</i>	SA4612 ^f	J (106)	80	35	D (800)	525	275	2,457
<i>cysA1367</i>	SA4601 ^f	K (240)	45	205	D (800)	600	200	2,527
<i>purC882</i>	SA4578 ^f	K (240)	102	147	D (800)	505	143, 155	2,585
<i>guaB554</i>	SA4607 ^f	K (240)	125	125	D (800)	680	120	2,607
<i>glyA540</i>	SA4579 ^f	K (240)	190	60	D (800)	745	55	2,673
<i>purG1739</i>	SA4610 ^f	K (240)	200	50	D (800)	755	45	2,683
<i>pheA534</i>	SA4628 ^f	L (48)	12	45	E (130)	8	122	2,736
<i>tyrA555</i>	SA4580 ^f	L (48)	15	42	E (130)	11	119	2,739
<i>tctA1511</i>	SA4605 ^f	M (647)	31	626	E (130)	74	56	2,802
<i>cysC1511</i>	SA4603 ^f	M (647)	167	490	F (415)	80	335	2,938
<i>argA1832</i>	SA4581 ^f	M (647)	227	430	F (415)	140	275	2,998
<i>lysA565</i>	SA4582 ^f	M (647)	265	395	F (415)	178	237	3,036
<i>argR372</i>	SA4606 ^g	M (647)	620	40	F (415)	115	35	3,390
<i>cysG1510</i>	SA4604 ^f	P (310)	35	282	F (415)	65	455	3,480
<i>ompR1009</i>	SA4632 ^g	P (310)	70	250	F (415)	NC	—	3,515
<i>xyl-183</i>	SA4586 ^f	P (310)	245	73	H (522)	265	255	3,690
<i>ilvA595</i>	SA4588 ^f	S (281)	17	275	I (281)	12	273	3,957
<i>cya-1091</i>	SA4595 ^g	S (281)	53	240	I (281)	48	238	3,993
<i>ntr-352</i>	SA4599 ^g	S (281)	110	180	I (281)	105	176	4,050
<i>metB879</i>	SA4589 ^g	S (281)	206	85	I (281)	200	81	4,145
<i>argH1823</i>	SA4590 ^f	S (281)	235	55	I (281)	230	50	4,175
<i>mel-351</i>	SA4591 ^f	A' (780)	130	660	J (290)	126	164	4,351
<i>purA</i>	SA4649 ^f	A' (780)	210	580	J (290)	—	—	4,431
<i>pyrB692</i>	SA4592 ^f	A' (780)	315	475	J (290)	NC	—	4,536
<i>serB695</i>	SA4593 ^f	A' (780)	405	385	(1,348)	110	1,230	4,626
<i>thr-557</i>	SA4570 ^f	A' (780)	435	355	(1,348)	140	1,218	4,656

Continued on the following page

TABLE 3. *Xba*I fragments from the genome of *S. paratyphi* B IP87/87

<i>Xba</i> I fragment ^a	Size		<i>Xba</i> I site at counterclockwise end			Gene at <i>Xba</i> I site ^f	Fragment recut ^g by:	
	kb ^b	CS ^c	No. ^d	Location ^e			<i>Ceu</i> I	<i>Bln</i> I
				kb	CS			
A	780	16.8	19	4,226	90.9	<i>gltV</i> (<i>rrnE</i>)	2.3 ('F), 780 (G')	290 (J), 490 (A')
B	272	5.8	1	350	7.5	<i>rrnH</i>	2.3 ('G), 270 (A')	272 ('A')
C	750	16.1	2	622	13.4		750 ('A')	585 ('A), 72 (B), 92 (C')
D	52	1.1	3	1,372	29.5		52 ('A')	52 ('C')
E	160	3.4	4	1,424	30.6		160 ('A')	160 ('C')
F	80	1.7	5	1,584	34.1		80 ('A')	80 ('C')
G	390	8.4	6	1,664	35.8		390 ('A')	265 ('C), 125 (D')
H	108	2.3	7	2,054	44.2		108 ('A')	108 ('D')
I	220	4.7	8	2,162	46.5		220 ('A')	220 ('D')
J	106	2.3	9	2,382	51.2		106 ('A')	106 ('D')
K	240	5.2	10	2,488	53.5		240 ('A), 2.3 (B')	240 ('D)
L	48	1.0	11	2,728	58.7	<i>gltW</i> (<i>rrnG</i>)	48 ('B')	48 (E')
M	647	14.0	12	2,776	59.7		650 ('B), 2.3 (C')	80 ('E), 415 (F), 150 (G)
N	20	0.4	13	3,423	73.6	<i>gltT</i> (<i>rrnD</i>)		
O	7	0.2	14	3,443	74.0			
P	310	6.6	15	3,450			310 ('C')	310 ('H')
Q	110	2.4	16	3,760	80.9			
R	75	1.6	17	3,870				
S	281	6.0	18	3,945		<i>gltU</i> (<i>rrnC</i>)	2.3 ('C), 92 (D), 145 (E), 42 (F')	280 (I)

^a See Table 1, footnote a.^b See Table 1, footnote b.^c See Table 1, footnote c.^d See Table 1, footnote d. For *Xba*I sites, numbering begins at site *Xba*I-1 on the clockwise side of fragment A at 7.5 CS.^e See Table 1, footnote e.^f See Table 1, footnote f.^g See Table 1, footnote g.

the hypothesis (13) that *nanH* entered *S. typhimurium* LT2 by lateral transfer and that the equivalent DNA is missing from other salmonellae.

Analysis of other *S. paratyphi* B strains. All 22 *S. paratyphi* B strains in the SARA set (4) were digested with *Xba*I and *Bln*I and separated by PFGE; the 14 strains from SARA41 to SARA54 are shown in Fig. 4. All five strains of electrophoretic type Pb3 (SARA50 to SARA54), including SARA52, which is IP87/87, the strain analyzed in detail in this report, show very similar fragment sizes. The same fragments of the same sizes are shown in Fig. 1B. Strains of other electrophoretic types show many bands of the same sizes, especially for *Xba*I, but also show considerable differences.

There was no evidence of a plasmid as part of the genome of strain IP87/87, since all of the DNA fragments were placed on the chromosome. This result was expected; a plasmid is usually present as part of the genome in some serovars but was not detected in *S. paratyphi* B (32).

DISCUSSION

Linkage maps show strong conservation between *E. coli* and *S. typhimurium* (37). In this report, we show that gene order is also conserved, as expected, in a comparison between *S. typhimurium* and *S. paratyphi* B. However, DNA segments have been lost or gained in the evolution of the genera *Escherichia* and *Salmonella*, producing a substantial number of strongly divergent regions (15, 34) apparently due to deletions or insertions of genetic material. We found evidence (discussed below) that evolution within the salmonellae has also involved deletions and insertions.

Genomic cleavage maps of *S. paratyphi* B and *S. typhimurium* LT2, determined through the locations of endonuclease sites and the locations of *Tn10* insertions in homologous genes, were compared (Fig. 3). The positions of homologous genes were determined in the two serovars through mapping of *rrn* genes (on the basis of *Ceu*I sites) or by use of *Xba*I and *Bln*I

^a Gene into which *Tn10* is inserted and allele number of the insertion. Mutations beginning with "z" are in a region of unknown function. Some strains have other mutations. The insertions are usually wild-type *Tn10* (TetR), but in some strains, they are *Tn10*dTet (*Tn10*Δ16Δ17).

^b *Tn10* in *S. typhimurium* was transduced into *S. paratyphi* B by use of phage P22 with selection for tetracycline resistance.

^c DNA from the strains of *S. paratyphi* B with *Tn10* insertions was digested with *Xba*I. The fragment which is missing and into which *Tn10* is inferred to be inserted is shown under "Normal," along with the size in kilobases. The two new fragments, from cleavage of the missing band in the *Tn10 Xba*I site, are indicated. Fragment A is counterclockwise, and fragment B is clockwise.

^d As in footnote c but for analysis following digestion with *Bln*I.

^e Location of *Tn10* on the chromosome of *S. paratyphi* B, in kilobases from the *thr* gene, calculated as described in the text. The position is the average from *Xba*I and *Bln*I analyses.

^f The phenotype associated with the *Tn10* insertion in *S. typhimurium* was observed after transduction into *S. paratyphi* B, indicating that *Tn10* had been inserted by homologous recombination into the same gene as in the donor. For example, *thr*-557::*Tn10* gave rise to a threonine-deficient phenotype in both *S. typhimurium* (parent strain TT191) and *S. paratyphi* B (SA4570).

^g Not tested for phenotype following *Tn10* transfer.

^h —, failure to produce analyzable data.

ⁱ NC, enzyme cutting was not detected.

TABLE 4. *BlnI* fragments from the genome of *S. paratyphi* B IP87/87

<i>BlnI</i> fragment ^a	Size		<i>BlnI</i> site at counterclockwise end			Gene at <i>BlnI</i> site ^f	Fragment recut ^g by:	
	kb ^b	CS ^c	No. ^d	Location ^e			<i>XbaI</i>	<i>CeuI</i>
				kb	CS			
A	1,348	30.0	10	4,516	97.0	<i>gltW</i> (<i>rrnG</i>)	490 ('A), 272 (B), 585 (C')	490 ('G), 855 (A')
B	72	1.5	1	1,208	25.9		72 ('C')	72 ('A')
C	648	14.0	2	1,280	27.5		90 (C'), 52 (D), 160 (E), 80 (F), 265 (G')	650 ('A')
D	800	17.2	3	1,928	41.4	<i>gltT</i> (<i>rrnD</i>)	125 ('G), 108 (H), 220 (I), 106 (J), 240 (K)	800 ('A), 2.2 (B')
E	130	2.8	4	2,728	58.6		48 (L), 80 (M')	130 ('B')
F	415	8.9	5	2,858	61.4		415 ('M')	415 ('B')
G	150	3.2	6	3,273	70.3	<i>gltU</i> (<i>rrnC</i>)	150 ('M')	50 ('B), 2.2 (C')
H	522	11.2	7	3,423	73.5		20 and 6.4 (N, O), 310 (P), 110 and 75 (Q, R)	520 ('C')
I	281	6.0	8	3,945	84.7		280 (S)	2.2 ('C) 92 (D), 145 (E), 42 (F')
J	290	6.2	9	4,226	90.8	<i>gltV</i> (<i>rrnE</i>)	290 ('A')	2.2 ('F), 290 (G')

^a See Table 1, footnote a.^b See Table 1, footnote b.^c See Table 1, footnote c.^d See Table 1, footnote d.^e See Table 1, footnote e.^f See Table 1, footnote f.^g See Table 1, footnote g.

sites in *Tn10* transposons; these homologous genes are joined by solid lines in Fig. 3, for their homology is proven; *XbaI* and *BlnI* sites in similar positions in the two serovars are joined only by broken lines, for their homology is inferred but not proven. The positions of 68 homologous genes were mapped (61 determined from *Tn10* transposons; 7 determined from *CeuI* sites in *rrn* genes), and no changes in gene order were detected, indicating that no major inversions or translocations have occurred. In most cases, the lengths of the intervals between the same genes in the two serovars are similar, e.g., the *thr-pyrA* interval is 78 kb in *S. typhimurium* and 75 kb in *S. paratyphi* B, suggesting that the genetic material between the two serovars is basically homologous. However, there is strong evidence for differences in interval lengths in some cases, as follows.

(i) The *nanH* region is inferred, for the following reasons, to include an insertion of DNA at 1,140 kb in *S. typhimurium*. The *nanH* gene and its enzyme activity are present in LT2 but missing from other *Salmonella* strains (13). The *pncB-pyrD* interval is 45 kb in LT2 but only a few kilobases in *S. paratyphi* B (Fig. 3 and Table 2). These same genes are not jointly transduced by P22 in *S. typhimurium* but are jointly transduced when the phage is grown on *S. paratyphi* B, indicating close proximity. The LT2 interval contains a *BlnI* site which is missing from *S. paratyphi* B (according to McClelland et al. [27], *BlnI* sites are rare in enteric bacteria but relatively common in many other bacterial species, suggesting lateral transfer).

(ii) The *rrnH* gene, indicated by the *CeuI*-1 site at 300 kb in *S. typhimurium*, appears to be shifted in *S. paratyphi* B. The *pan-proA* interval is almost identical in both serovars (150 kb in LT2 and 160 kb in *S. paratyphi* B), but the locations of the

CeuI-1 site within this interval are very different (69 kb from *proA* in LT2 but only 28 kb in *S. paratyphi* B).

(iii) The *tyrA-cysC* interval in LT2 is 266 kb, while the homologous interval in *S. paratyphi* B is 199 kb. The longer LT2 interval contains three extra *XbaI* sites clustered into a small region. The sequence CTAG, found in both *XbaI* and *BlnI* sites, occurs rarely in enteric bacteria (27), apparently because these sites are removed from DNA mismatches by the *vsr* gene product (5, 11). These data suggest that the region in LT2 containing the three *XbaI* sites was recently acquired from a species which lacks the *vsr* gene product; the CTAG sites have not yet been deleted by the *vsr* system.

Whereas the locations of genes on the genomic cleavage maps of *S. typhimurium* and *S. paratyphi* B are strongly conserved, *XbaI* and *BlnI* restriction sites are often not conserved. An analysis of Fig. 3 shows that there are 24 *XbaI* sites in *S. typhimurium* and 19 in *S. paratyphi* B; 13 of these are inferred to be at homologous locations, 4 because they are in *Glt*-tRNA genes in *rrn* genes and 9 because they are in similar locations (indicated by broken lines in Fig. 3). However, 11 sites in *S. typhimurium* and 6 in *S. paratyphi* B have no obvious homologs in the other serovar, indicating that in *S. typhimurium*, over half of the *XbaI* sites outside the *rrn* genes are not present in *S. paratyphi* B. There are 12 *BlnI* sites in *S. typhimurium* and 10 in *S. paratyphi* B; of these, only 6 are homologous, 4 in *Glt*-tRNA genes and 2 at other apparently homologous sites (*BlnI*-3 and *BlnI*-5 sites in *S. paratyphi* B). In *S. typhimurium*, four *BlnI* sites are in the genes for 16S rRNA (*rrs* genes) (*BlnI*-8, *BlnI*-9, *BlnI*-11, and *BlnI*-12 sites), while in *S. paratyphi* B, there are no *BlnI* sites in the *rrs* genes (Fig. 3); there are two other nonhomologous sites in *S. typhimurium* (*BlnI*-2 and *BlnI*-6 sites) and four in *S. paratyphi* B (*BlnI*-1,

FIG. 3. Comparison of the genomic cleavage maps of *S. typhimurium* LT2 and *S. paratyphi* B IP87/87. The positions of the cleavage sites for *XbaI* (●), *BlnI* (○), and *CeuI* (□) and of the genes of *S. typhimurium* were determined in kilobases beginning at *thr*, based on data presented earlier (22) and on recent studies (unpublished data). The positions of sites and genes in *S. paratyphi* B are from this report. A horizontal line joins points on the two separate maps if they are known or inferred to be homologous.

FIG. 4. Fingerprints of the genome of strains of *S. paratyphi* B determined by *Xba*I and *Bln*I digestion and separation by PFGE. The strains are from the SARA set assembled by Beltran et al. (4); 41 to 54 refer to their SARA numbers. Strain IP87/87 (SARA52) is studied in detail in this report. Pb1 and Pb1a, etc., refer to the electrophoretic types determined by multilocus enzyme electrophoresis analysis (4); IP87/87 belongs to Pb3 electrophoretic type. In a few cases, the sizes of fragments have been rounded to the nearest 10.

*Bln*I-2, *Bln*I-6, and *Bln*I-10 sites). However, it is not certain that the sequences underlying the *Bln*I sites are not conserved, for Perkins et al. (30) detected four *Bln*I targets in nucleotide sequences (one in *rrsD*) which are not cleaved in any of the six different *E. coli* K-12 strains which they examined. This result indicates that *Bln*I sequences are sometimes not cleaved, perhaps because of nucleotide modifications.

In contrast to the lack of conservation of *Xba*I and *Bln*I sites, all seven *Ceu*I sites are conserved between *S. typhimurium* and *S. paratyphi* B (Fig. 3), as reported earlier (21). This fact is hardly surprising, since the *Ceu*I cleavage site in the chloroplast DNA encoding the large-subunit rRNA of *C. eugamatos* is a large site (up to 26 bp), and this site is completely conserved in other chloroplast and mitochondrial DNAs and also in the *rrlB* gene of *E. coli* K-12 (10), as well as in many other rRNA sequences from prokaryotes, as determined by a BLAST (1) search of GenBank sequences. This high degree of conservation is common in rRNA sequences (42), and it is not surprising that it also extends to all seven *rrl* genes in salmonellae.

Thus, the numbers and locations of *rrn* genes are strongly conserved between *S. typhimurium* and *S. paratyphi* B (Fig. 3).

The rearrangement which places Glt-tRNA-encoding sequences in *rrnD* in *S. typhimurium* (22) and in *S. enteritidis* (23) rather than in *rrnB* as in *E. coli* (17) is also found in *S. paratyphi* B (Fig. 3). As noted above, the *Bln*I site which is present in the *S. typhimurium* *rrs* gene for 16S rRNA (and discovered in the *rrnB* sequence in *E. coli* [6, 29]) is not present in any of the seven *rrs* genes of *S. paratyphi* B. An *Xba*I site (*Xba*I-1 site) is present in the *rrnH* gene of *S. paratyphi* B, 2.3 kb counterclockwise of the *Ceu*I-1 site; this is the same distance as that between the *Ceu*I and *Xba*I sites in other *rrn* genes, such as *rrnC*, in which the *Xba*I site is inferred to be in the Glt-tRNA gene. It is very surprising to find an *Xba*I site at this location, because of the conservation of sequences in *rrn* genes; this finding may be the result of homologous recombination with some other *rrn* gene which has an *Xba*I site at this location. Exchanges between *rrn* genes are relatively common (12). *Xba*I sites at this location, inferred to be in the Glt-tRNA gene, are accompanied by a *Bln*I site in *E. coli* (30), *S. typhimurium* (22), *S. enteritidis* (23), and *S. paratyphi* B (*Xba*I-11, *Xba*I-13, *Xba*I-18, and *Xba*I-19 sites; this report).

The pattern of ethidium bromide-stained fragments following digestion of bacterial genomic DNA and PFGE (the

fingerprint of a strain) can be used as a measure of the relatedness of bacteria. *XbaI* and *BlnI* digestion of 22 strains of *S. paratyphi* B from the SARA set of reference strains (4) revealed almost complete identity for the fingerprint patterns of the five strains of electrophoretic type 3 (Fig. 4); SARA51, SARA53, and SARA54 are human isolates from France, but SARA50 is from food from the Middle East and SARA52 is from a cow in France. This conservation of fingerprint patterns of these strains from very diverse sources, which indicates near identity of the locations of *XbaI* and *BlnI* sites, is strong evidence affirming that the strains belonging to electrophoretic type Pb3 are actually part of a clone, as proposed by Selander et al. (41). It is obvious that changes in *XbaI* and *BlnI* sites occur fairly rapidly, since *S. typhimurium* and *S. paratyphi* B have diverged significantly, but no divergence has occurred within electrophoretic type Pb3 of *S. paratyphi* B. However, other electrophoretic types of *S. paratyphi* B show significant changes in fingerprint patterns, a fact which fits with their placement in different clones (41).

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