Molec. gen. Genet. 139., 177—188 (1975)

DNA Restriction and Modification Systems in *Salmonella*

III. SP, a *Salmonella potsdam* System Allelic to the SB System in *Salmonella typhimurium*

Leonard R. Bullas* and Charles Colson

Laboratoire de Cytogénétique, Louvain-La-Neuve, Belgium

Received February 10, 1975

Summary. By screening 42 *Salmonella* strains with P3, a temperate bacteriophage with an unusually wide host range, five new DNA restriction and modification systems (R-M systems) were identified in five different serotypes in Kauffmann-White group C. One of these systems, SP, in a Pl-sensitive strain of *S. potsdam*, was analyzed genetically by Pl transduction methods in which SP was transferred into *S. typhimurium* and *E. coli/S. typhimurium* hybrids. It was found that the genes of the SP system were allelic and functionally homologous to the genes of the SB system of *S. typhimurium*.

Introduction

Host specificity for DNA is a bacterial property determined by *hsd* genes located on the bacterial chromosome or on a cell plasmid whose biological detection is based on a non-hereditable, host-imposed change in the host-range properties of suitable infecting bacteriophages. Thus, in *Salmonella typhimurium*, phages P22 and L are used to recognize the *S. typhimurium* R-M systems LT (Colson, Colson and Van Pel, 1969, 1970) and SA (Colson and Colson, 1971; Colson and Van Pel, 1974). A third system in *S. typhimurium* designated SB was recognized in a unique way. Its genes were transferred from *S. typhimurium* to *E. coli* during the construction of *E. coli/S. typhimurium* hybrids (Colson and Colson, 1971). This system was detected in these hybrids by a change in the host-range properties of phage λ (Colson and Van Pel, 1974). It is determined by genes allelic and functionally homologous to those of the K and B systems of *Escherichia coli* (Van Pel and Colson, 1974).

In view of the many well characterized *Salmonella* serotypes available, we found it of interest to investigate whether strains other than *S. typhimurium* might also harbor genes for the R-M system SB or for R-M systems related to SB.

In this paper we describe a method of screening for *Salmonella* systems and have analyzed genetically one system in *Salmonella potsdam* which was found to be allelic to the *S. typhimurium* SB system. This screening method utilized phage P3 which was isolated originally from a lysogenic culture of *S. potsdam* (Atkinson and Bullas, 1956) and has a host range which includes many

* This work was done while on sabbatical leave at the Université de Louvain. Present address: Department of Microbiology, School of Medicine. Loma Linda University, Loma Linda, California 92354, U.S.A.

Table 1. *Salmonella* serotypes screened for sensitivity to lysis by P3

Kauffmann-White group	Name	Antigenic formula	Source
A	*S. paratyphi* A	1, 2, 12; a; —.	A
B	*S. brandenburg* (5)[a]	1, 4, 5, 12; l, v; e, n, z_{15}	B
	S. derby (2)	1, 4, 5, 12; f, g; —.	B
	S. haifa	1, 4, 5, 12; z_{10}; 1, 2.	B
	S. schwartzengrund	1, 4, 12, 27; d; 1, 7.	B
C_1	*S. ardwick*	6, 7, 14; f, g; —.	D
	S. cholerae-suis var. Kunzendorf	6, 7; —; 1, 5.	D
	S. eimsbuettel	6, 7, 14; d; l, w.	D
	S. gelsenkirchen	6, 7, 14; l, v; z_6.	D
	S. infantis	6, 7; r; 1, 5.	C
	S. isangi	6, 7; d; 1, 5.	B
	S. kaduna	6, 7, 14; c; e, n, z_{15}.	D
	S. oranienburg	6, 7; m, t; —.	D
	S. potsdam	6, 7; l, v; e, n, z_{15}.	C
	S. menston	6, 7; g, s, t; —.	B
	S. thompson (7)	6, 7; k; 1, 5.	B and C
C_2	*S. muenchen*	6, 8; d; 1, 2.	C
D	*S. blegdam*	9, 12; g, m, q; —.	C
	S. dublin (3)	1, 9, 12; g, p; —.	C
	S. eastbourne	1, 9, 12; e, h; 1, 5.	C
	S. enteritidis (5)	1, 9, 12; g, m; —.	B and C
	S. gallinarum	1, 9, 12; —; —.	C
	S. panama	1, 9, 12; l, v; 1, 5.	C
	S. typhosa	9, 12; d; —.	A
E	*S. anatum*	3, 10; e, h; 1, 6.	C

[a] Number in parentheses following the name of the *Salmonella* refers to the number of strains tested.

A = From the Department of Microbiology, Loma Linda University, Loma Linda, California, U.S.A. B = From the Institute of Hygiene and Epidemiology, Brussels, Belgium. C = From the Department of Microbiology, University of Adelaide, Adelaide, Australia. D = From the Center for Disease Control, Atlanta, Georgia, U.S.A.

different serotypes of *Salmonella* and *E. coli* (Nutter, Bullas and Schultz, 1970). In addition, this phage was found also to be subject to host-induced modification (Nutter, Bullas, Siapco and Pearson, 1972). Thus phage P3 was considered as potentially a good phage for use as an indicator for other unidentified R-M systems in *Salmonella* and *E. coli*.

Materials and Methods

Bacteria and Bacteriophages. The different *Salmonella* serotypes investigated, with their sources, are listed in Table 1. The strain of *S. potsdam* is the rough mutant used by Nutter, Bullas, Siapco and Pearson (1972) derived from the strain originally used by Atkinson and Bullas (1956). A *leu* mutant of *S. potsdam* designated L1002 was derived by treatment with N′-N-methyl-N-nitrosoguanidine (NG). The various strains of *E. coli*, *S. typhimurium* and *E. coli*/*S. typhimurium* hybrids with their origins are listed in Table 2.

Table 2. Strains of *E. coli*, *S. typhimurium* and *E. coli*/*S. typhimurium* hybrids

Strain number	Host specificity phenotype	Genotype	Origin
1.	*Escherichia coli* K-12		
1228	$r_K^- m_K^-$	*serB*80 *leu thi lac* hsd_K	from *E. coli* K 12, strain C600, Colson and Van Pel (1974)
2.	*Salmonella typhimurium* LT2		
4247	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB* str^r	SL1027 from B.A.D. Stocker
4274	$r_{LT}^- m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val* str^r hsd_{LT}	from 4247 with NG[a]
4278	$r_{LT}^- m_{LT}^- r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val* str^r hsd_{LT}	from 4247 with NG
4411	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+$	*proC*90 F′-*lac*$^+$ hsd_{LT} hsd_{SA}	from *proC*90 from K. Sanderson
4414	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^- r_{SB}^+ m_{SB}^+$	*metA metB trpB val* str^r hsd_{LT} hsd_{SA}	from 4247 with NG
4419	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val* str^r hsd_{LT} hsd_{SA}	from 4247 with NG
4512	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*serB*80	from K. Sanderson
4514	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*serB*80 *galE*	from 4512; spontaneous FO resistant[b]
4516	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*serA*13 *pyr*124	P22 co-transduction of *pyr*124 from 4510 (Colson and Van Pel, 1974) to an *arg*I derivative of HfrK4 *serA*13 from K. Sanderson
4519	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*serA*13 *thr*17	from HfrK4 *serA*13 with NG
4520	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*serA*13 *thr*25	from HfrK4 *serA*13 with NG
4522	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*leu pyrB*124	as 4516 but with HfrK19 from K. Sanderson
4526	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metE trpB val gal* str^r hsd_{LT} hsd_{SA}	from 4419 with FO selection
4529	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	F′-*gal*$^+$ *bio*	SL1694 from B.A.D. Stocker
4530	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val pro gal* hsd_{LT} hsd_{SA}	from 4526 with NG
4532	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val pyrB*124 *gal*	from 4522 × 4530 cross
4534	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val thr*25 *gal*	from 4520 × 4530 cross
4536	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA metB trpB val serB*80 *gal*	P1 transduction of *serB*80 from 4514 to 4534

[a] NG is the mutagen N′-N-methyl-N-nitrosoguanidine.
[b] FO is phage FO.

Table 2. (continued)

Strain number	Host specificity phenotype	Genotype	Origin
3.	*E. coli*/*S. typhimurium hybrids*[c]		
4617	$r^{+}_{SA}\ m^{+}_{SA}\ r^{+}_{SB} m^{+}_{SB}$	(*serB*80$^-$ *leu*$^+$) *thi lac*	Colson and Van Pel, 1974
4652	$r^{+}_{SA}\ m^{+}_{SA}\ r^{-}_{SB}\ m^{-}_{SB}$	(*serB*80$^-$ *leu*$^+$ hsd_{SB}) *thi lac*	from 4617 with NG
4655	$r^{+}_{SA}\ m^{+}_{SA}\ r^{-}_{SB}\ m^{+}_{SB}$	(*serB*80$^-$ *leu*$^+$ hsd_{SB}) *thi lac*	from 4617 with NG

[c] Markers in brackets are those from *S. typhimurium*.

The *vir* mutant of phage λ, and phages P1, P22 and L were the same as previously used (Colson and Colson, 1971; Colson and Van Pel, 1974). Phage P3 was originally isolated from a lysogenic culture of *S. potsdam* and is the one used by Nutter, Bullas, Siapco and Pearson (1972).

Bacteriophage lysates of phage λ and P1 were prepared by extraction from soft agar layers, with added $MgSO_4$ at 0.01 M for phage λ, and with added $CaCl_2$ at 0.01 M for P1. Lysates of P22 and L were prepared by propagation on the appropriate hosts in aerated L broth. Phage P3 was similarly propagated in L broth but with the addition of $CaCl_2$ at a concentration of 1.25×10^{-2} M if the propagating strain was *E. coli*. No $CaCl_2$ was necessary with a *Salmonella* as host. EDTA to a final concentration of 1 mg/ml was added to lysates of P3 to stabilize. Stocks of phages λ, P1, P22 and L were sterilized with chloroform; those of phage P3 by Millipore filtration.

Notations. The notations for host specificity phenotypes and genotypes, and those for phage modifications, follow the recommendations or Arber and Linn (1969), Arber (1974) and Colson and Van Pel (1974). The restriction-modification system (R-M system) in *S. potsdam* described in this paper was designated SP.

Media. All the media were the same as used by Colson and Van Pel (1974).

Mutagenesis. Cells in the exponential phase of growth were centrifuged, resuspended in phosphate buffer at pH 6.0 and treated with 30 μg/ml of N′-N-methyl-N-nitrosoguanidine (NG) for 30 min at 37 °C. NG-treated cells were washed twice in buffer, diluted in broth and incubated overnight. These cultures were plated out on suitable media, incubated 24–48 hrs to obtain isolated colonies and then scored.

Conjugation Crosses. Two ml of an unshaken exponential culture of the donor strain were mixed with one ml of a shaken exponential culture of the recipient strain and kept at 37 °C for two hours. The mixed culture was then centrifuged and the cells resuspended in 0.5 ml of buffer and the total contents plated out on medium selective for the desired recombinants.

Transductions. The use of phage P1 for transduction in *S. typhimurium* and *S. potsdam* was possible because (a) rough, *gal* derivatives of *S. typhimurium* SL1027 were P1-sensitive and (b) the rough *S. potsdam* strain used was sensitive to phage P1. Accordingly, P1 propagated on the donor strain was added to exponential broth cultures of the recipient strain at a multiplicity of infection of 1 in the presence of 0.01 M $CaCl_2$ and left at room temperature for 30 min. The cells were then centrifuged and resuspended in buffer containing 0.05% sodium citrate and plated out on selective medium containing 0.05% sodium citrate, according to the method of Glover (1962).

Recombinants derived from *S. typhimurium* were made *gal*$^+$, thus restoring sensitivity to phages P22 and L, by cross-streaking with F′-*gal*$^+$ strain 4529, on selective medium containing galactose instead of glucose. Recombinants were purified by streaking out on selective medium before being tested further.

Measure of Efficiency of Plating (e.o.p.) of Phages. The e.o.p. of a phage was measured by plating it out by the usual agar layer method on both a strain with no detectible R-M system (e.g. 1228) and on other suitable indicator strains. The e.o.p. was the number of plaques on the indicator strain relative to the number of plaques on the strain with no detected R-M system.

Approximate e.o.p. was determined by spotting out various dilutions of phages on these same strains.

Tests to Determine Restriction and Modification Phenotypes. These tests were performed on purified cultures of recombinants. The spot test and the cross-streaking tests were those described by Colson, Colson and Van Pel (1970). Test phages for *E. coli* and *E. coli*/*S. typhimurium* hybrids were λ and P3, and for *S. typhimurium*, P3, P22 and L. Phage L was included because of its special sensitivity to the SA system (Colson and Van Pel, 1974).

Isolation of Restriction Deficient Mutants of S. potsdam. Restriction deficient mutants of *S. potsdam* were isolated following treatment with NG. The mutagenized cultures were cross-streaked with F′-*lac*$^+$ strain 4411. *Lac*$^+$ colonies growing on selective medium were purified and tested for their restriction phenotype with phage P3 propagated on *S. potsdam* and with P3.LT, SB. Those *lac*$^+$ cultures which failed to restrict P3.LT, SB had the r^+_{SP} phenotype.

P3 plaques on r^-_{SP} cultures were tested for modification by plating them out with wild-type *S. potsdam*. Modification mutants (m^-_{SP}) were restricted by *S. potsdam* while m^+_{SP} mutants were not restricted by *S. potsdam*. Of the host modification mutants isolated, two were selected for use in the work in this paper—one $r^-_{SP}m^+_{SP}$ mutant designated L1007 and one $r^-_{SP}m^-_{SP}$ mutant designated L1004.

Results

A. Effect of the S. typhimurium R-M Systems on Phage P3

On most strains of *S. typhimurium* LT2 and LT7, P3 produced only minute plaques. On SL1027 and its derivatives, however, P3 produced its typical large, round and temperate plaques. On these derivatives it was therefore possible to produce high titer lysates of P3 bearing the different modifications of the R-M systems already recognized in *S. typhimurium*. Since, at the start of these investigations, the SB system had not yet been recognized, all P3 lysates possessed the SB modification. P3 bearing also the LT modification was prepared by propagation on strain 4414, P3.SA, SB was prepared by propagation on strain 4278 and P3.LT, SA, SB by propagation on 4274. P3 lacking all known modifications (P3.0) was prepared by propagation on *E. coli* 1228. The e.o.p. of each of these lysates was measured on indicator strains for the LT, SA and SB systems as well as on 1228. These results are shown in Table 3.

Table 3. Efficiency of plating of P3 with different modifications on strains of *S. typhimurium* with different R-M phenotypes

Strain number	R-M phenotype	Efficiency of plating of phage[a]			
		P3.0 (1228)	P3.LT, SA, SB (4247)	P3.LT, SB (4414)	P3.SA, SB (4278)
E. coli					
1228	$r^-_K\ m^-_K$	1	1	1	1
S. typhimurium					
4247	$r^+_{LT}\ m^+_{LT}\ r^+_{SA}\ m^+_{SA}\ r^+_{SB}\ m^+_{SB}$	2×10^{-7}	1	1	2×10^{-7}
4419	$r^-_{LT}\ m^+_{LT}\ r^-_{SA}\ m^+_{SA}\ r^+_{SB}\ m^+_{SB}$	1.5×10^{-2}	1	1	1
4278	$r^-_{LT}\ m^-_{LT}\ r^+_{SA}\ m^+_{SA}\ r^+_{SB}\ m^+_{SB}$	4×10^{-2}	1	1	1

[a] Propagating strains are given in parentheses.

Table 4. The efficiency of plating of P3.SA, SB and P3.LT, SB on P3-sensitive *Salmonella* strains

Kauffmann-White group	*Salmonella*	e.o.p. of phage	
		P3.SA, SB	P3.LT, SB
A	*S. paratyphi* A	1.2×10^{-7}	1
C_1	*S. cholerae-suis*	3.9×10^{-7}	1
	S. gelsenkirchen	2.8×10^{-7}	2.1×10^{-2}
	S. kaduna	7.5×10^{-7}	9.1×10^{-3}
	S. oranienburg	1.4×10^{-7}	8.8×10^{-3}
	S. potsdam	1.0×10^{-7}	6.5×10^{-5}
C_2	*S. muenchen*	2.0×10^{-7}	3.0×10^{-5}
D	*S. typhosa*	1	1
	S. dublin (3 strains)	1.5×10^{-6}	1

Several conclusions may be drawn concerning the sensitivity of P3 to these systems. 1. Since P3.LT, SB had an e.o.p. of 1 on strains 4278 (r^+_{SA}) and 4419 (r^-_{SA}) P3 is insensitive to SA restriction. 2. Since P3.SA, SB had an e.o.p. of 2×10^{-7} on 4247 (r^+_{LT}) P3 is sensitive to LT restriction. 3. Since P3.0 had an e.o.p. of about 10^{-2} on 4419 and 4278 (both r^+_{SB}) while P3.SA, SB was not restricted, P3 is sensitive to SB restriction.

B. Detection of R-M Systems in a Variety of Salmonella Serotypes

All the *Salmonella* listed in Table 1 were first tested for lysis by phage P3. Of these 42 cultures, 11 were sensitive to P3. The e.o.p. of P3.SA, SB and P3.LT, SB on each of these strains were determined (Table 4).

Clearly, the P3-sensitive *Salmonella* fall into three groups: Group 1 which consists of those strains restricting neither P3.SA, SB nor P3.LT, SB; these strains must not have LT restriction ability; the only member of this group is *S. typhosa*. Group 2 which consists of those strains restricting P3.SA, SB but not restricting P3.LT, SB; these strains must have LT restriction ability and include *S. paratyphi* A, *S. cholerae-suis* and the three strains of *S. dublin*. Group 3 consisting of those strains restricting strongly P3.SA, SB and to lesser degree, P3.LT, SB; these strains must have LT restriction ability and another restriction and include *S. potsdam*, *S. oranienburg*, *S. kaduna*, *S. gelsenkirchen* and *S. muenchen*. All the members of this third group are found in the Kauffmann-White serological group C distinguished by the possession of somatic antigen 6.

In order to test whether the restrictions of P3 observed in the five group C *Salmonella* were caused by five independent R-M systems, P3 was propagated on each of these five *Salmonella* strains and plated out on each of the other *Salmonella* to determine their e.o.p. The results of these tests are shown in Table 5.

When P3 was propagated on any one of these five *Salmonella* strains, it was restricted by each of the other four. Thus each of the five serotypes has one or more R-M systems with unique specificity. Further work in this paper concerns the genetic analysis of the R-M system in *S. potsdam*, which we designated SP.

Table 5. The efficiency of plating of P3 propagated on each of the *Salmonella* strains possessing a new specificity, on each other

Culture	e.o.p. of P3 propagated on				
	S. gelsenkirchen	*S. kaduna*	*S. oranienburg*	*S. potsdam*	*S. muenchen*
S. gelsenkirchen	1	3.3×10^{-3}	1.4×10^{-2}	8.0×10^{-3}	2.8×10^{-2}
S. kaduna	4.6×10^{-3}	1	6.9×10^{-4}	1.3×10^{-3}	7.7×10^{-3}
S. oranienburg	1.2×10^{-2}	1.5×10^{-2}	1	7.2×10^{-3}	1.0×10^{-2}
S. potsdam	2.5×10^{-5}	6.0×10^{-5}	2.8×10^{-4}	1	1.0×10^{-4}
S. muenchen	4.5×10^{-5}	2.4×10^{-4}	2.1×10^{-4}	4.0×10^{-5}	1

C. Preliminary Mapping of the hsd_{SP} Genes by Conjugation

As a first step in attempting to locate the region of the chromosome where hsd_{SP} might be located, we thought it best to assume that the locus might be near one of the regions where already identified *hsd* genes had been located in *S. typhimurium*. We assumed that the *S. potsdam* chromosome was essentially similar to that of *S. typhimurium*. Since we had already detected the LT system in *S. potsdam* (which is close to the *proC* locus in *S. typhimurium*) we investigated the possibility that hsd_{SP} might be in the *pyrB–serB* region where the two other *S. typhimurium* systems, SA and SB, were located.

A number of preliminary conjugation experiments was done employing the *leu str*^{r} mutant of *S. potsdam*, L1002, as recipient in conjugation with *S. typhimurium* Hfr strains 4516 (*serA* pyr_{124}.) and 4519 (*serA* thr_{17}) as donors. Selection was for *leu*$^+$ *str*^{r} recombinants with *thr* or *pyr* as unselected markers. The restriction status of recombinants was determined with the use of P3 bearing appropriate modifications.

These crosses proved to be only weakly fertile. Although significant numbers of *pyr* recombinants were obtained from the cross involving Hfr 4516, only a very few *thr* recombinants were obtained from the cross involving Hfr 4519. Of nine *leu*$^+$ *pyr*$^-$ recombinants, six were r^-_{SP}, and of four *leu*$^+$ *thr*$^-$ recombinants, two were r^-_{SP}. Thus, although only a few recombinants were obtained, these results did indicate that the hsd_{SP} genes were probably located on the chromosome and in the *pyrB–serB–leu* region.

D. P1 Transduction Mapping of hsd_{SP} in S. typhimurium Recipients

The *S. typhimurium* strains 4532 and 4536 were used as recipients for *pyrB*$^+$ and *serB*$^+$ from *S. potsdam*, respectively, in P1-mediated transduction. The presence of hsd_{SP} was determined by means of spot tests employing phages P3, P22 and L.

Phage L was included, principally since it was a good indicator for hsd_{SA} (Colson and Colson, 1971). The results of these tests giving the approximate e.o.p. of each phage on the different *hsd* types of *pyrB*$^+$ recombinants isolated from the transduction with 4532 as recipient, and their inferred phenotypes, are shown in Table 6. These results indicate that both phages P22 and L are subject to SP restriction. Four groups of *hsd pyrB*$^+$ recombinants were defined. The first group, consisting of 75% of the *pyrB*$^+$ recombinants, had the same *hsd* genotype

Table 6. The approximate efficiencies of plating of phages P3, P22 and L on *pyrB*$^+$ recombinants resulting from P1 transduction from *S. potsdam* to 4532 (hsd^+_{LT} hsd^+_{SA} hsd^+_{SB} *pyr*)

Recombinant group	Frequency of recombinants	Efficiency of plating of phage[a]				Inferred restriction phenotype
		P3.LT, SA, SB (4419)	P3."P" (*S. potsdam*)	P22.LT, SA, SB (4419)	L.LT, SB (4414)	
1	124/166	1	10^{-2}	1	10^{-2}	r^+_{SA} r^+_{SB} r^-_{SP}
2	35/166	1	10^{-2}	1	1	r^-_{SA} r^+_{SB} r^-_{SP}
3	6/166	10^{-2}	1	10^{-2}	10^{-2}	r^-_{SA} r^-_{SB} r^+_{SP}
4	1/166	10^{-2}	1	10^{-2}	10^{-4}	r^+_{SA} r^-_{SB} r^+_{SP}

[a] Propagating strains are given in parentheses.

Table 7. Transduction mapping of hsd_{SA} and hsd_{SP} genes by P1-mediated co-transduction with *pyrB* and *serB* in *S. typhimurium*

Donor	Recipient	Selected donor marker	Total number tested	Number with recombinant phenotype			Co-transduction frequency	
				r^+_{SP} r^+_{SA}	r^+_{SP} r^-_{SA}	r^+_{SB} r^-_{SA}	hsd^+_{SP}	hsd^-_{SA}
S. potsdam	4532	*pyrB*$^+$	166	1	6	35	4.2%	25%
S. potsdam	4536	*serB*$^+$	172	26	4	1	17%	2.9%

as the recipient hsd^+_{SA} hsd^+_{SB} hsd^-_{SP}. The other three groups were recombinant for these three *hsd* loci. Thus, the second group, consisting of 21% of *pyrB*$^+$ recombinants, had the genotype hsd^-_{SA} hsd^+_{SB} hsd^-_{SP}, the third group consisting of 3.6% of *pyrB*$^+$ recombinants, had the genotype hsd^-_{SA} hsd^-_{SB} hsd^+_{SP} and the fourth group, consisting of only one recombinant or 0.6% had the genotype hsd^+_{SA} hsd^-_{SB} hsd^+_{SP}.

These data confirmed that hsd_{SP} was on the chromosome and in the *pyrB* region. Since 25% of the *pyrB*$^+$ recombinants were hsd^-_{SA}, hsd_{SA} was missing in *S. potsdam* and the 25% frequency represented the co-transduction linkage frequency of hsd_{SA} and *pyrB*. The hsd_{SP} genes present in 4.2% of *pyrB*$^+$ recombinants represented the co-transduction linkage frequency of hsd_{SP} to *pyrB*.

Another highly significant result was the failure to isolate any recombinants that were hsd^+_{SB} hsd^+_{SP} since all recombinants were either hsd^+_{SB} hsd^-_{SP} or hsd^-_{SB} hsd^+_{SP}. In this respect, therefore, hsd_{SB} and hsd_{SP} behaved as alleles.

A similar P1 transduction experiment to *S. typhimurium* 4536 was performed using P1 propagated on *S. potsdam* as vector and selecting for *serB*$^+$ recombinants. In this experiment also, no recombinants which had gained hsd^+_{SP} retained hsd^+_{SB}. In Table 7 are summarized the gene mapping data with both 4532 and 4536 recipients with selection for *pyrB*$^+$ and *serB*$^+$, respectively.

These data are compatible only with the gene order *pyrB*–hsd_{SA}–(hsd_{SB}/hsd_{SP})–*serB* in which hsd_{SB} and hsd_{SP} are alleles.

Table 8. Transduction mapping of hsd_{SA} and hsd_{SP} genes by P1-mediated co-transduction with *serB* in *E. coli*/*S. typhimurium* hybrid 4617

Donor	Recipient	Selected donor marker	Total number tested	Number with recombinant phenotype			Co-transduction frequency	
				$r^+_{SP}\ r^+_{SA}$	$r^+_{SP}\ r^-_{SA}$	$r^+_{SB}\ r^-_{SA}$	hsd^+_{SP}	hsd^-_{SA}
S. potsdam	4617	*serB*+	230	61	15	1	33%	6.9%
L4003	4617	*serB*+	708	281	—	—	40%	—

L4003 is a *serB*+ hsd^+_{SA} hsd^+_{SP} recombinant derived from the first cross with *S. potsdam*.

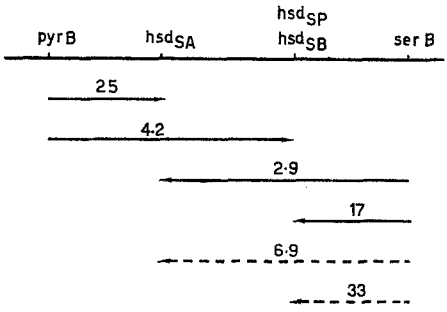

Fig. 1. P1 co-transduction frequencies with *pyrB* or *serB* of hsd_{SA}, hsd_{SB} and hsd_{SP}. Arrows point towards the unselected markers. Unbroken lines: transduction in *S. typhimurium*. Dashed lines: transduction in hybrids

E. Transduction Mapping of hsd_{SP} in E. coli/S. typhimurium Hybrid 4617

P1 propagated on *S. potsdam* was also used as vector to transduce *serB*+ to the *E. coli*/*S. typhimurium* hybrid 4617. *SerB*+ recombinants were examined for host specificity with phage λ bearing the SB or SP modifications. The results of this experiment are presented in Table 8.

These results indicate that hsd_{SA} was co-transduced with *serB* at a frequency of 6.9% and that hsd_{SP} was co-transduced with *serB* at a frequency of 33%. These data also confirm the absence of hsd_{SA} in *S. potsdam* and confirm the order of loci deduced from the transduction data with *S. typhimurium*.

Also presented in Table 8 is the result of a P1 co-transduction experiment to 4617 in which P1 was propagated on one of the hsd^+_{SA} hsd^+_{SP} *serB*+ recombinants (designated L4003) derived from the transduction experiment in which P1 had been propagated on *S. potsdam*. In this experiment, there was a 40% co-transduction frequency linkage between hsd_{SP} and *serB*. This slightly higher co-transduction frequency linkage between these two loci (i.e. 40% instead of 33% with the earlier experiment) probably rests on the fact that P1 propagated on *S. potsdam* lacks the SA modification whereas P1 propagated on L4003 had the SA modification. Thus the transducing DNA derived from *S. potsdam* was subject to SA restriction to which DNA derived from L4003 was immune.

A linkage map of the co-transduction data in both *S. typhimurium* and the *E. coli*/*S. typhimurium* hybrid is given in Fig. 1.

Table 9. Recombination between hsd_{SB} and hsd_{SP} mutants in P1-mediated co-transduction with $serB^+$ in *E. coli*/*S. typhimurium* hybrids

Donor		Recipient	Total tested	Frequency of	
				$r^+_{SB}\ m^+_{SB}$ phenotype	$r^+_{SP}\ m^+_{SP}$ phenotype
L1004	$r^-_{SP}\ m^-_{SP}$	4655 $r^-_{SB}\ m^+_{SB}$	166	2 (1.2%)	0
S. potsdam					
	$r^+_{SP}\ m^+_{SP}$	4655 $r^-_{SB}\ m^+_{SB}$	136	2 (1.4%)	26 (19%)
L1007	$r^-_{SP}\ m^+_{SP}$	4652 $r^-_{SB}\ m^-_{SB}$	401	0	3 (0.75%)

F. Functional Homology between hsd_{SB} and hsd_{SP}

Functional homology between R-M systems can be demonstrated: (a) by showing that complementation occurs in merodiploids harboring mutated genes in both systems (Boyer, Rolland-Dussoix, 1969; Glover, 1970); and (b) by obtaining hsd^+ recombinants in crosses performed between mutants in the two systems (Glover and Colson, 1969).

A similar series of transduction experiments to those of Glover and Colson (1969) was done to test the functional homology between hsd_{SB} and hsd_{SP} genes. In these experiments, selection was always for $serB^+$ recombinants; the recipients were either the $r^-_{SB}\ m^+_{SB}$ hybrid 4655 or the $r^-_{SB}\ m^-_{SB}$ hybrid 4652; the donors were wild-type *S. potsdam* and the $r^-\ m^+$ mutant L1007 and the $r^-_{SP}\ m^-_{SP}$ mutant L1004. $SerB^+$ recombinants were purified from a series of three transductions and first tested for restriction by cross-streaking with λ.SB and λ.SP. All recombinants that were r^+_{SB} or r^+_{SP} were further tested to check that they also had the corresponding modification properties. Results of these experiments are summarized in Table 9. In each of the three crosses, hsd^+ recombinants were obtained whose origin is most simply explained by crossing over within a three gene R-M system—*hsdM* (for modification), *hsdS* (for specificity) and *hsdR* (for restriction).

These results demonstrate that there is functional homology between hsd_{SB} and hsd_{SP} genes and they are consistant with the generally accepted idea that the specificity of *hsd* systems resides in the *hsdS* gene (see Meselson, Yuan and Heywood, 1972) mutations of which lead to the production of $r^-\ m^-$ phenotypes.

Discussion

Our results demonstrate that several R-M systems exist among different *Salmonella* serotypes, each with a unique specificity. Although the recognition of these systems was confined to a set of P3-sensitive *Salmonella*, it is clear that this property need not be a requirement for future investigations. The only prerequisite for this analysis is the availability of P1-sensitive mutants for each *Salmonella* serotype being investigated. Ornellas and Stocker (1974) have shown that some classes of rough mutants of *S. typhimurium* are P1-sensitive, as was, by

chance, the rough strain of *S. potsdam* used here. It is possible to isolate P1-sensitive mutants from members of the family *Enterobacteriaceae* (Goldberg, Bender and Streicher, 1974). Thus the methods are now available to demonstrate the presence of R-M systems linked to *pyrB* and *serB* in any *Salmonella* strain. We have recently tested the general applicability of the method to P3-insensitive *Salmonella* strains and have demonstrated the presence of such a system in one strain.

The system SP, in *S. potsdam*, is allelic and functionally homologous to the SB system in *S. typhimurium* recently reported by Colson and Van Pel (1974). Whether the R-M systems detected with P3 in the other Kauffmann-White group C *Salmonella* have the same chromosomal location as SP has yet to be fully determined but preliminary results suggest that this is indeed so.

Although no positive evidence of a functional homology between SP and the K and B systems of *E. coli* has yet been demonstrated, the observations that SB has functional homology with these systems (Van Pel and Colson, 1974) and that SP is functionally homologous with SB strongly suggest that the SP system may be functionally homologous with the *E. coli* systems.

The demonstration of functional homology between the SP and the SB systems is also additional evidence in support of the "at-least-three-gene" model proposed for the *E. coli* K and B systems (see Arber and Linn, 1969). Our results also support the gene order of *hsdM–hsdS–hsdR–serB* proposed by Bulkacz (1972, cited by Arber, 1974). The existence of a multi-allelic series of R-M systems in *Salmonella*, functionally related to each other and to the *E. coli* K and B systems, would offer additional opportunities for further clarification of the genetic structure of these systems.

Of additional interest, is the observation that *S. typhosa* was the only *Salmonella* without LT restriction. We have since found that another seven, independently isolated strains of *S. typhosa* demonstrated a similar lack of LT restriction. This property would therefore appear to be a general characteristic of this organism. In light of the unique pathological characteristics of *S. typhosa*, one is lead to wonder if the lack of LT may be of some significance in this regard. The distribution of restriction abilities among the other P3-sensitive *Salmonella* is also interesting. The group 2 strains which demonstrated LT restriction only, *S. paratyphi* A, *S. cholerae-suis* and *S. dublin*, are all responsible for an enteric fever-like infection in man (see Wilson and Miles, 1955). The remaining *Salmonella*, placed into group 3, which demonstrated at least one restriction in addition to LT, and *S. typhimurium* with three known systems (LT, SA and SB), are all primarily responsible for gastro-enteritis in man.

There is therefore an apparent relationship between the severity or type of the disease caused by a particular *Salmonella* serotype and the nature of its restriction properties. Arber (1974) has commented that because of the widespread existence of R-M systems among the bacteria, "it might be important for bacteria to have restriction activity". If a definite relationship between restriction activity (or the lack of it) and disease potential of an organism could be shown to exist, the study of R-M systems would acquire medical significance.

References

Arber, W.: DNA modification and restriction. Progress in Nucleic Acid Research and Molecular Biology **14**, 1–37 (1974)

Arber, W., Linn, S.: DNA modification and restriction. Ann. Rev. Biochem. **38**, 467–500 (1969)

Atkinson, N., Bullas, L. R.: Lysogenicity and lysis patterns in the Salmonellas 10. *S. potsdam*. Aust. J. exp. Biol. med. Sci. **34**, 461–470 (1956)

Boyer, H. W., Roulland-Dussoix, D.: A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. molec. Biol. **41**, 459–472 (1969)

Colson, A. M., Colson, C., Van Pel, A.: Host-controlled restriction mutants of *Salmonella typhimurium*. J. gen. Microbiol. **58**, 57–64 (1969)

Colson, C., Colson, A. M.: A new *Salmonella typhimurium* DNA host specificity. J. gen. Microbiol. **69**, 345–351 (1971)

Colson, C., Colson, A. M., Van Pel, A.: Chromosomal location of host specificity in *Salmonella typhimurium*. J. gen. Microbiol. **60**, 265–271 (1970)

Colson, C., Glover, S. W., Symonds, N., Stacey, K. A.: Thel location of the genes for host-controlled modification and restriction in *Escherichia coli* K-12. Genetics **52**, 1043–1050 (1965)

Colson, C., Van Pel, A.: DNA restriction and modification systems in *Salmonella* I. SA and SB, two *Salmonella typhimurium* systems determined by genes with a chromosomal location comparable to that of the *Escherichia coli hsd* genes. Molec. gen. Genet. **129**, 325–337 (1974)

Glover, S. W.: Valine resistant mutants of *Escherichia coli*. Genet. Res. **3**, 448–460 (1962)

Glover, S. W.: Functional analysis of host specificity mutants in *Escherichia coli*. Genet. Res. **15**, 237–250 (1970)

Glover, S. W., Colson, C.: Genetics of host-controlled restriction and modification in *Escherichia coli*. Genet. Res. **13**, 227–240 (1969)

Goldberg, R., Bender, R. A., Streicher, S. L.: Direct selection for Pl-sensitive mutants of enteric bacteria. J. Bact. **118**, 810–814 (1974)

Meselson, M., Yuan, R., Heywood, J.: Restriction and modification of DNA. Ann. Rev. Biochem. **41**, 447–466 (1972)

Nutter, R. L., Bullas, L. R., Schultz, R. L.: Some properties of five new *Salmonella* bacteriophages. J. Virol. **5**, 754–764 (1970)

Nutter, R. L., Bullas, L. R., Siapco, B. S., Pearson, C. A.: Change in methylation of *Salmonella* bacteriophage P3 deoxyribonucleic acid with host-controlled modification by *Escherichia coli*. J. Virol. **10**, 560–562 (1972)

Ornellas, E. P., Stocker, B. A. D.: Relation of lipopolysaccharide character to P1 sensitivity in *Salmonella typhimurium*. Virology **60**, 491–502 (1974)

Van Pel, A., Colson, C.: DNA restriction and modification system in *Salmonella* II. Genetic complementation between the K and B systems of *Escherichia coli* and the *Salmonella typhimurium* system SB with the same chromosomal location. Molec. gen. Genet. **135**, 51–60 (1974)

Wilson, G. S., Miles, A. A.: Topley and Wilson's principles of bacteriology and immunity, 4th ed. Baltimore, U.S.A.: The Williams and Wilkins Company 1955

Communicated by W. Arber

Dr. Leonard R. Bullas
Department of Microbiology
Loma Linda University
Loma Linda, California 92354
USA

Dr. Charles Colson
Laboratoire de Cytogénétique
Place de la Croix du Sud, 4
B-1348 Louvain-La-Neuve
Belgium