Host-controlled Restriction Mutants of *Salmonella typhimurium*

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SUMMARY

Forty-eight independent restriction-deficient mutations of *Salmonella typhimurium* LT2 were isolated by using selective and non-selective methods. With phage P 22 it was shown that some mutations affected the restriction capacity only, while others affected both restriction and modification. The host-restriction of *S. typhimurium* decreased the recovery of F-*lac*⁺ infected cells and decreased the yield of recombinants in bacterial mating and in phage P 22-mediated transduction.

INTRODUCTION

Host specificity is a process by which bacterial cells control the acceptance of foreign DNA. Two strain-specific mechanisms are involved in the achievement of this control. (1) The DNA synthesized within the cell can be altered in a non-inheritable fashion (*modification*). In at least one case this modification has been shown to be the methylation of a DNA base (Arber & Smith, 1966). (2) Upon entry into the cell, foreign DNA which does not carry the appropriate modification can be rapidly degraded (*restriction*). The primary step in this degradation is due to a specific endonuclease which causes a discrete number of double-chain scissions in the DNA (Meselson & Yuan, 1968). Strain-specific sites, probably base sequences, along the DNA, must be present and recognized before either restriction or modification can be exercised (Arber & Kühnlein, 1967).

Most of our present understanding of host specificity comes from genetical and biochemical studies in *Escherichia coli* in which differences in host specificity between strains were described several years ago and in which it was possible to isolate and study host specificity deficient mutants (review: Arber & Linn, 1969). *Salmonella typhimurium* LT2 and LT7 strains are extensively used in genetical studies, yet no difference in host specificity has been described between them. The existence in *S. typhimurium* of a barrier to the acceptance of DNA carrying *E. coli* host specificity was nevertheless suggested by some incidental observations (Zinder, 1960; Miyake, 1962). Furthermore, it has been shown that the fertility (*fer*) mutation in *S. typhimurium* results in the loss of previously undetected host restriction towards the DNA of *E. coli* (Colson & Colson, 1967; Okada, Watanabe & Miyake, 1968). The key observation in these studies was that the restriction which affects *E. coli* DNA also affects phage P 22 DNA. Most *fer* mutants lose both the restriction and the modification functions, and lysates of phage P 22 produced by them form plaques less efficiently (10^{-4}) when plated on *S. typhimurium* LT7 or LT2 than on the *fer* mutants themselves.

The experiments presented in this paper constitute the first step in an investigation of the genetic control of the host specificity that was revealed in *Salmonella typhimurium* by the isolation of *fer* mutants. We describe the isolation and the phenotypic characterization of a collection of restriction deficient mutants from *S. typhimurium* strain LT2.

METHODS

Bacteria. *Salmonella typhimurium* strains LT7 and LT7 *mut* (carrying a mutator gene) were obtained from G. G. Meynell (Lister Institute, London). Restriction deficient mutants of these strains were described previously (Colson & Colson, 1967). *S. typhimurium* LT2: *proC* 90 and *proC* 110 were obtained from H. O. Smith; SR305 HfrA *hisD* 23 *gal*-50 and SB106 *ile*-405 *rha*-461 *leu*-1003 *proA* 46 *purC* 7 *str-r* from P. Hartman; SA534 HfrK 4 *serA* 13, SA195 *proAB* 47 *purE* 66, and *argD* 6 from K. Sanderson; SL1027 *metA metE trpB xyl str-r* from B. Stocker.

Escherichia coli K12; W1655 *met lac F-lac*⁺; a derivative of C600 lacking the K host specificity (Colson, Glover, Symonds & Stacey, 1965). *E. coli* B: a derivative of BC251 lacking the B host specificity (Glover & Colson, 1969).

Bacteriophages. Phage P 22 wild type was obtained from P. Fredericq. A *c* 2 mutant was provided by G. G. Meynell. P 22 *int-11*, lacking a function necessary for the integration of the prophage into the bacterial chromosome (Smith & Levine, 1967) was donated by H. O. Smith.

Media. Nutrient broth contained Difco Bacto Tryptone, 10 g.; NaCl, 8 g.; distilled water, 1 l. Nutrient agar was nutrient broth solidified with 1.5% (w/v) Difco Bacto agar. Phage agar was broth containing 1% (w/v) Difco Bacto agar and was used as bottom layer for phage assay in soft agar overlays. Soft agar was 0.6% (w/v) Difco Bacto agar in distilled water. EMB lactose agar, was as Arber & Morse (1965). Minimal medium (g./l.): NH₄Cl, 5; NH₄NO₃, 1; Na₂SO₄, 2; K₂HPO₄, 3; KH₂PO₄, 1; MgSO₄·7H₂O, 0.1; this medium was solidified with 1.5% (w/v) Difco Bacto agar and supplemented with 0.02 g./l. of appropriate L-amino acids or adenine when necessary. Dilution buffer contained (g./l.): KH₂PO₄, 3; Na₂HPO₄, 7; NaCl, 4; MgSO₄·7H₂O, 0.2.

Mutagenic treatment. Exponentially growing broth cultures were incubated at 37° for 25 min. in the presence of *N'*-*N*-methyl-*N*-nitrosoguanidine (NTG) 30 µg./ml. NTG was removed by twice washing in buffer. The cells were then diluted 1/100 in fresh broth and incubated to allow expression of the mutations.

Terminology. According to Arber & Linn (1969) the following terminology for host specificity will be used. The host specificity system of *Salmonella typhimurium* LT7 and LT2 will be noted as 'LT'. The phenotypes will be characterized as r_{LT} for restriction and m_{LT} for modification, with the superscript plus or minus. Thus $r_{LT}^{+}m_{LT}^{+}$ characterizes wild type, while $r_{LT}^{-}m_{LT}^{+}$ and $r_{LT}^{-}m_{LT}^{-}$ will indicate respectively strains lacking the LT restriction, and both the LT restriction and the LT modification. The presence or the absence of the LT modification on phage P 22 will be specified by the notation for the LT specificity preceded by a point. Thus P 22.LT characterizes phage P 22 carrying the LT modification, while P 22.o indicates phage P 22 lacking any detectable modification.

RESULTS

Isolation of restriction—deficient mutants

Stocks of phage P 22 *c* 2 lacking the LT modification (P 22 *c* 2.0) were obtained on a previously isolated *fer* mutant of *Salmonella typhimurium* LT7 (Colson & Colson, 1967) and on an *argD* mutant of strain LT2 that was found to lack both restriction and modification. A wide variation in efficiency of plating (e.o.p.) was observed when these phage stocks were plated on NTG-treated cultures of LT2. On most cultures the phage formed plaques at a higher frequency than on control cultures. It was logical to assume that the increase in successful infections originated from infection of restriction-deficient mutants, thus raising the e.o.p. of P 22.0 significantly higher than that obtained under normal condition of restriction. This test was therefore repeated on a large number of independent NTG-treated cultures from several strains of LT2 to assess the efficiency of the mutagenic treatment and to discriminate those cultures which after further selection could be expected to yield mutants. An increase in e.o.p. from 10^{-4} to more than 10^{-3} was commonly observed, indicating the presence of more than 0.1 % of accepting cells. Such powerful mutagenesis allowed simple methods to be used with success for the isolation of the mutants. About 1×10^8 cells from the NTG-treated cultures were spread on nutrient agar plates together with 1×10^5 particles of P 22 *c* 2.0. After overnight incubation, the plates were screened for the presence of colonies showing phage infection at their edges. Such colonies were picked, restreaked and tested for their restriction phenotype. About one-third of them were found to be r_{LT}^- (40 out of 126 tested). Most mutants were isolated by this method, but two other selective methods were used.

It was shown that *F-lac* is subject to host-restriction when transferred from *Escherichia coli* to *Salmonella typhimurium* (Colson & Colson, 1967). Transfer could therefore select accepting cells, as does the transfer of an R factor (Okada & Watanabe, 1968). Samples of mutagen-treated cultures of *S. typhimurium* were spread on minimal lactose plates together with samples of exponentially growing *E. coli* w 1655 *F-lac*⁺*met*. Contra-selection of the donor was achieved by omitting methionine from the medium and by the presence of streptomycin when a *met* recipient strain was used (SL1027). Some r_{LT}^- were found among the *lac*⁺ colonies, but only at a frequency of 10 % (6 of 68 tested).

Transduction of bacterial markers from a strain lacking modification was also used as a method to select r_{LT}^- mutants in the recipient. Preliminary experiments had revealed that the frequency of transduction by P 22 was very poor in LT7 when the transducing lysate was grown on LT7 $r_{LT}^-m_{LT}^-$ (Colson & D'Aout, unpublished). However, the fact that most P 22 transduced clones are immune to P 22 under the conditions used meant that scoring for restriction with P 22.0 was impossible. This difficulty was overcome with a P 22 *int* mutant (Smith & Levine, 1967). Such phage mutants are deficient in the integration function and cannot lysogenize by themselves. Lysogenic strains for P 22 *int* can nevertheless be obtained by complementation between *int* and *c* mutants. After induction, such lysogenic cultures produce highly defective lysates which transduce some bacterial markers (*proC*, *purE*) at an abnormally high frequency (Smith, 1968). LT2 *argD* 6 $r_{LT}^-m_{LT}^-$ was lysogenized with P 22 *int-11* and induced according to the methods of Smith (1968). The lysate was utilized to transduce *proC*⁺ and *purE*⁺ to NTG-treated strains. Most transduced clones were

found to be P 22-sensitive after one restreaking. Only a few of them (5 of 195 tested) were found to be restriction-deficient.

To ensure that all the mutants for further studies originated from independent mutational events only one r_{LT}^- clone was kept from each NTG treated culture. A total of 48 independent restriction-deficient mutants were isolated in LT2 strains, 37 were obtained from direct screening, 6 after F-*lac*⁺ infection and 5 by P 22 *int*.0 transduction.

Table 1. *Host specificity phenotypes among 48 restriction-deficient mutants of Salmonella typhimurium*

Strain	No. of mutants with phenotype			
	$r_{LT}^-m_{LT}^-$	$r_{LT}^-m_{LT}^+$	$r_{LT}^+m_{LT}^+$	$r_{LT}^-m_{LT}^\pm$
SR 305	0	1	0	1
SA 534	3	1	0	5
SB 106	0	2	0	0
SA 195	2	2	1	4
SL 1027	1	1	0	3
<i>proC</i> 90	9	3	0	4
<i>proC</i> 110	3	0	0	2
Total	18	10	1	19

Restriction ability was determined by plating samples of serial dilutions of P 22.0 with samples of exponentially growing cultures of the mutants. r_{LT}^- corresponds to an e.o.p. of about 1.0; r_{LT}^+ to an e.o.p. of 10^{-1} . Modification ability was determined by picking and resuspending phage from a single plaque on each mutant and plating serial dilutions of it on the mutant itself and on wild type LT 2. Mutants producing phage with the same e.o.p. on both strains were classified as m_{LT}^+ , those from which the phage was restricted about 10^{-4} were scored as m_{LT}^- , those producing phage that was restricted 10^{-1} to 10^{-3} were scored as $m_{LT}^\pm$.

Phenotypic classes of restriction-deficient mutants

Several host specificity phenotypes were observed among the restriction-deficient mutants. These phenotypic classes were distinguished by the degrees of acceptance of P 22.0 and from the subsequent e.o.p. of the phage produced by the mutants when plated on wild-type hosts. Table 1 summarizes the frequency of these classes among the various strains. A weak restriction was found in only one mutant ($r_{LT}^-m_{LT}^+$) in which P 22.0 was restricted about 10-fold. Among the others some had completely lost the ability to confer the modification ($r_{LT}^-m_{LT}^-$) while others produced fully modified P 22 ($r_{LT}^+m_{LT}^+$). Quite frequently intermediate abilities to modify were observed. Phage produced by these mutants plated on wild-type hosts with an e.o.p. between 10^{-1} and 10^{-3} . These phenotypes intermediate for modification were classified as $r_{LT}^-m_{LT}^\pm$. Table 2 shows the e.o.p. of P 22 on LT2 and on some of its r_{LT}^- mutants and also on LT7 and on some LT7 mutants isolated previously (Colson & Colson, 1967). These results confirm that there is no difference in host specificity between LT2 and LT7. When phage was grown on wild type LT2 its e.o.p. on LT7 was as good as on LT2, and vice versa. Similarly, when the phage was grown on a m_{LT}^- mutant of either LT2 or LT7 its e.o.p. was decreased by a similar factor on both wild-type strains. The decreased restriction which is commonly observed in LT7 *mut* is caused by a high frequency of mutator-induced r_{LT}^- mutants in the population.

Host restriction of bacterial DNA

Host specificity mutants were isolated in several genetically marked strains and in Hfr donor strains. It was therefore possible to investigate the incidence of restriction in bacterial matings and in transduction. Pairs of otherwise isogenic r_{LT}^+ and r_{LT}^- strains were used as recipients in conjugation experiments with two Hfr strains, one of which carried a mutation leading to the $r_{LT}^- m_{LT}^-$ phenotype. Within each set of four crosses, restriction of donor DNA could occur in only one mating pair (Hfr

Table 2. *Efficiencies of plating of P 22 on LT 2, LT 7 and r_{LT}^- mutants of Salmonella typhimurium*

Host strain	Phage lysates prepared from				
	LT 7	LT 7 $r_{LT}^- m_{LT}^-$	LT 2	LT 2 $r_{LT}^- m_{LT}^+$	LT 2 $r_{LT}^- m_{LT}^-$
	Relative e.o.p.				
LT 7	1	4.0×10^{-4}	1	1	1.6×10^{-4}
LT 7 $r_{LT}^- m_{LT}^-$	1	1	1	1	1
LT 7 <i>mut</i>	1	6.4×10^{-4}	1	1	1.0×10^{-3}
LT 2	1	3.0×10^{-4}	1	1	1.5×10^{-4}
LT 2 $r_{LT}^- m_{LT}^+$	1	1	1	1	1
LT 2 $r_{LT}^- m_{LT}^-$	1	1	1	1	1

Phage from a single plaque of P 22 c 2 grown on each host strain was used to inoculate the same host in soft agar overlays. After incubation, the overlays were harvested, shaken with chloroform and buffer and cleared by centrifugation. The lysates were suitably diluted and plated in overlays with exponentially growing cultures of each strain. The e.o.p. values given are based on counts of several hundred plaques. Differences in titre of less than 20% were not regarded as significant.

Table 3. *Host restriction in bacterial matings in Salmonella typhimurium*

Recipient strain	Selected marker	No. of recombinants per donor cell		Corrected restriction value
		$r_{LT}^+ m_{LT}^+$	$r_{LT}^- m_{LT}^-$	
SB 106	<i>proA</i> ⁺	2.4×10^{-3}	2.4×10^{-4}	5.1×10^{-2}
SB 106 $r_{LT}^- m_{LT}^+$	<i>proA</i> ⁺	4.5×10^{-3}	2.3×10^{-3}	
SB 106	<i>leu</i> ⁺	4.0×10^{-3}	3.0×10^{-4}	4.1×10^{-2}
SB 106 $r_{LT}^- m_{LT}^+$	<i>leu</i> ⁺	7.4×10^{-3}	4.0×10^{-3}	
<i>proC</i> 90	<i>proC</i> ⁺	5.0×10^{-4}	2.9×10^{-5}	1.5×10^{-1}
<i>proC</i> 90 $r_{LT}^- m_{LT}^-$	<i>proC</i> ⁺	8.0×10^{-5}	1.8×10^{-4}	

The donor strains were SA 534 HfrK 4 (order of transfer: *leu-pro-purE*) and a $r_{LT}^- m_{LT}^-$ derivative of it. Exponentially growing cultures of the donor strains were diluted 1/10 into prewarmed broth and mixed with equal volumes of exponentially growing cultures of the recipient strains. The mixtures were centrifuged and placed at 37° in a water bath, without resuspending the pellets. After 60 min. the mixtures were shaken vigorously, diluted and plated on selective media. All selections were made in the presence of streptomycin 100 µg./ml., to which only the donors were sensitive. The donor input was estimated from the colony count on nutrient agar plates seeded with diluted samples of the donor cultures at the start of the matings.

$r_{LT}^- m_{LT}^- \times F^- r_{LT}^+ m_{LT}^+$). The degree of restriction was estimated from the difference in the frequency of recombinants obtained between the two crosses in which $r_{LT}^+ m_{LT}^+$ and $r_{LT}^- m_{LT}^-$ donors were mated with the same $r_{LT}^+ m_{LT}^+$ recipient strain. Errors resulting from possible variations in fertility between individual donor cultures were corrected

for from the difference in the number of recombinants for a given marker obtained in two control crosses in which the same two donor cultures were mated with a single non-restricting recipient strain. The number of recombinants in $Hfr\ r_{LT}^-m_{LT}^- \times F^-r_{LT}^+m_{LT}^+$ crosses was clearly decreased as compared to the yield of the control crosses (Table 3).

The same kind of experiments were performed to assess the restriction of *F-lac*. *F-lac*⁺ donors were obtained in LT2 and LT2 $r_{LT}^-m_{LT}^-$ after mating with *Escherichia coli* K12 W1655 *F-lac*⁺. Strain W1655 *F-lac*⁺ was also used as donor and restriction-deficient mutants of *E. coli* K12 and B were included among the recipients. The yield of *lac*⁺ clones was decreased about 100-fold in those crosses in which restriction could be exercised (Table 4). In the absence of restriction, transfer of *F-lac*⁺ is not more effective between LT2 strains than between *E. coli* and LT2. It appears also that restriction-deficient mutants of *E. coli* are even better recipients for *F-lac*⁺ transferred from LT2 than LT2 itself.

Table 4. *Host restriction of an F-lac⁺ factor in Salmonella typhimurium and Escherichia coli*

Recipient strain	No. of <i>lac</i> ⁺ clones per <i>F-lac</i> ⁺ donor			Corrected restriction value
	W 1655 <i>F-lac</i> ⁺	LT2 <i>F-lac</i> ⁺	LT2 $r_{LT}^-m_{LT}^-$ <i>F-lac</i> ⁺	
<i>proC</i> 90	6.4×10^{-7}	8.5×10^{-5}	4.1×10^{-6}	} 6.2×10^{-2}
<i>proC</i> 90 $r_{LT}^-m_{LT}^-$	2.1×10^{-4}	1.6×10^{-4}	2.1×10^{-4}	
SL 1027	3.2×10^{-7}	2.7×10^{-4}	2.3×10^{-6}	} 1.2×10^{-2}
SL 1027 $r_{LT}^-m_{LT}^-$	1.3×10^{-4}	2.7×10^{-4}	3.8×10^{-4}	
<i>E. coli</i>				
B 251 $r_B^-m_B^-$	—	4.4×10^{-4}	1.3×10^{-3}	—
C 600 $r_K^-m_K^-$	—	8.5×10^{-4}	2.0×10^{-3}	—

Procedure as in Table 3, except that the male input was measured by plating samples of the donor on EMB lactose medium to exclude the *lac* segregants.

Table 5. *Host restriction in P 22-mediated transduction of Salmonella typhimurium*

Recipient strain	Selected marker	No. of transductants per 10^{10} plaque-forming units		Corrected restriction value
		P 22.LT	P 22.O	
<i>proC</i> 90	<i>proC</i> ⁺	3.9×10^4	3.0×10^3	} 3.0×10^{-3}
<i>proC</i> 90 $r_{LT}^-m_{LT}^-$	<i>proC</i> ⁺	4.5×10^4	2.0×10^4	
<i>proC</i> 110	<i>proC</i> ⁺	9.8×10^4	5.4×10^3	} 4.6×10^{-3}
<i>proC</i> 110 $r_{LT}^-m_{LT}^-$	<i>proC</i> ⁺	4.9×10^4	4.2×10^4	
SB 106	<i>proA</i> ⁺	4.8×10^3	2.2×10^2	} 5.1×10^{-2}
SB 106 $r_{LT}^-m_{LT}^+$	<i>proA</i> ⁺	5.0×10^3	5.5×10^3	
SA 195	<i>purE</i> ⁺	1.2×10^5	1.4×10^2	} 4.1×10^{-3}
SA 195 $r_{LT}^-m_{LT}^-$	<i>purE</i> ⁺	1.0×10^5	3.4×10^4	
SL 1027	<i>trpB</i> ⁺	7.3×10^3	1.3×10^1	} 1.1×10^{-3}
SL 1027 $r_{LT}^-m_{LT}^-$	<i>trpB</i> ⁺	6.2×10^3	3.8×10^3	

P 22 lysates containing about 1×10^{11} plaque-forming u./ml. were obtained by the agar layer method on prototrophic LT7 and LT7 $r_{LT}^-m_{LT}^-$ strains. Exponentially growing cultures of the recipient strains were centrifuged and the deposit suspended in buffer at about 1×10^9 cells/ml. 1×10^{10} P22 particles were added to the suspensions and kept for 15 min. at room temperature before plating on selective media.

A similar set of experiments, in which the frequency of transduction was measured for several markers with P 22.LT and P 22.o lysates in r_{LT}^+ and r_{LT}^- strains showed that the degree of restriction for bacterial markers in transduction was about 100-fold (Table 5).

DISCUSSION

The isolation and phenotypic characterization of a set of 48 restriction-deficient mutants in *Salmonella typhimurium* LT2 clearly shows that the genetic control of host specificity in *S. typhimurium* is similar to other host specificity systems that have been studied. Similar types of mutants have been obtained in phage P1 (Glover, Schell, Symonds & Stacey, 1963), in an extensive analysis in *Escherichia coli* K12 and B (Wood, 1966) and in *Pseudomonas aeruginosa* (Rolfe & Holloway, 1968). Host restriction in *Salmonella typhimurium* was found to affect the frequency of recombinants formed in bacterial matings, the frequency of transduction, and also the acceptance of plasmids, in accord with previous observations in *E. coli* (Boyer, 1964; Arber, 1964; Arber & Morse, 1965; Copeland & Bryson, 1966).

It seems therefore that similar mechanisms of control over the acceptance of foreign DNA might be widespread among bacteria and since many experiments involve the transfer of DNA between unrelated bacterial strains, such as those which investigate genetic homology between bacterial species and the possibility of plasmid transfer, in interpreting such experiments the role of host restriction should be borne in mind. A striking example of this was the *fer* mutation of *Salmonella typhimurium* which remained puzzling for some years until it was shown that *fer* mutants were restriction-deficient (Colson & Colson, 1967; Okada, Watanabe & Miyake, 1968).

The genetic basis of the mutations which affect host specificity cannot be inferred directly from the observed phenotypes. In particular, the fact that a single mutation quite often affects both restriction and modification remained unexplained until recently. Progress in understanding the mechanism of host specificity was achieved when it was shown that restriction and modification activities are exercised at particular sites on the DNA, which can be rendered an inadequate substrate for *both* activities by mutation (Arber & Kühnlein, 1967). Recombination and complementation studies lead to the demonstration of a third cistron in addition to restriction and modification (Glover, 1968; Glover & Colson, 1969; Arber & Linn, 1969). Mutations which affect host specificity can therefore occur in any one of at least three genes for which the symbols *hss*, *hsr* and *hsm* have been recently proposed (Arber & Linn, 1969). It is likely that among the mutants described in the present paper, those with an $r_{LT}^-m_{LT}^+$ phenotype lack the restriction function (*hsr*) while those with an $r_{LT}^-m_{LT}^-$ phenotype are deficient in the recognition of the sites (*hss*). A possible explanation for the $r_{LT}^-m_{LT}^+$ phenotype is a mutation in the *hss* gene resulting in a lowered affinity for the host specific sites on the DNA. At first sight it would appear that such mutations should affect the degrees of restriction and modification equally, but it must be pointed out that the assay systems for restriction and modification are not equally sensitive. In assaying restriction of P 22.o as much as 20% of the phage could be restricted and remain undetected as a significant change in efficiency of plating, while in assaying modification as little as 0.1% modified phage in a lysate can easily be detected.

In view of the extensive homology between the genetic maps of *Escherichia coli* and *Salmonella typhimurium* (Sanderson & Demerec, 1965) and the similarity between the

host specificity system investigated here and those of *E. coli*, it is attractive to think that host specificity in *S. typhimurium* may belong to the allelic series of host specificities that are found in *E. coli* and which map close to *thr*. (Boyer, 1964; Colson *et al.* 1965; Glover & Colson, 1969). Experiments to be published will show that this *S. typhimurium* host-specificity system has quite a different chromosomal location close to the *pro* region.

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