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Salmonella typhimurium LT2 Strains Which are r^- m^+ for all Three Chromosomally Located Systems of DNA Restriction and Modification

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We describe the derivation of two strains of *Salmonella typhimurium* LT2 which are r^- m^+ for all three of the known chromosomal genes for the restriction and modification of DNA, *hsdLT*, *hsdSA*, and *hsdSB*; the strains were designated LB5000 and LB5010. LB5000 is a smooth derivative sensitive to phage P22; LB5010 is a *galE* strain sensitive to phage P1.

Since the role of DNA restriction-modification (R-M) systems of *hsd* genes has been elucidated, recipient cells for unmodified or foreign DNA are usually restrictionless mutants. Such mutants may either modify and have the phenotype r^- m^+ or fail to modify and have the phenotype r^- m^-. Each R-M system consists of three genes: *hsdS* for specificity, *hsdR* for restriction, and *hsdM* for modification (1).

Salmonella typhimurium LT2 possesses three chromosomal systems of *hsd* genes: *hsdLT*, *hsdSA*, and *hsdSB* (5). The *hsdLT* genes have been precisely mapped between *proAB* and *proC* (C. Digneffe, Ph.D. thesis, University of Louvain, Louvain-la-Neuve, Belgium, 1977), whereas the *hsdSA* and *hsdSB* systems both have been mapped close to *serB* (5). Although strains with mutations of the *hsdLT* and *hsdSA* genes have been available for years (5), no strain was available in which there were mutations in all three systems of *hsd* genes.

The presence of the *hsdSB* genes on the *S. typhimurium* chromosome was detected only by using *Salmonella* phage P3, which can infect cells of both *S. typhimurium* and *Escherichia coli* (6). Phage P3, which lacks SB modification, is restricted by both r_{SB}^+ cells of *S. typhimurium* LT2 and by *E. coli* K-12 to which the *hsdSB* genes have been transferred by transduction. Each of phages P22, L, and P3 is sensitive to LT restriction (3), phage L alone is sensitive to SA restriction (5), and phage P3 alone is sensitive to SB restriction (2). Thus, all three phages are necessary for the recognition of all three of the *hsd* genes in *S. typhimurium* LT2.

We derived two strains of *S. typhimurium* LT2, designated LB5000 and LB5010, which are r^- m^+ for all three DNA R-M systems of *hsd* genes. LB5000 is a smooth strain, sensitive to phages P22, L, and P3; LB5010 is a *galE* derivative of LB5000 which is sensitive to phage P1. Bacteria used are listed in Table 1.

Since the Colson strain of *S. typhimurium* LT2, 4419, was already r^- m^+ for both the LT and the SA *hsd* genes, we isolated an r_{SB}^- m_{SB}^+ mutant of 4419 after additional treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) (4).

We screened for r_{SB}^- mutants by infecting the NG-treated cells with the F *lac*$^+$ plasmid from *E. coli* AB1874, which fails to confer SB modification, anticipating that the F *lac*$^+$ DNA would contain at least one SB specificity site and thus be restricted by r_{SB}^+ cells but not by r_{SB}^- cells. Thus r_{SB}^- mutants could be indirectly selected by examining Lac$^+$ colonies derived from the NG-treated, F *lac*$^+$-infected cells of strain 4419. Accordingly, exponential-phase cells of strain AB1874 grown at 37°C were mixed with exponential-phase cells of the NG-treated strain 4419 culture grown at 30°C at a donor-to-recipient ratio of 1:10, placed without shaking at 30°C for 30 min, plated out on MacConkey lactose agar containing 100 μg of streptomycin per ml, and incubated for 3 days at 37°C.

Of 68 Lac$^+$ colonies tested, 9 were shown to be r_{SB}^- by using phage P3. These mutants were further examined with phages P22 and L to verify that they were still r_{LT}^- and r_{SA}^-. One clone that lost F *lac*$^+$ spontaneously was selected and designated LB5000.

The results of spot tests of LB5000 for the determination of the restriction phenotype (3) are shown in Table 2. Since LB5000 failed to restrict P22 lacking LT modification, phage L lacking SA modification and phage P3 lacking SB modification, LB5000 had the restriction phenotype r_{LT}^- r_{SA}^- r_{SB}^-.

To test for modification, single plaques of P22, L, and P3 on LB5000 were suspended in buffer, diluted, and spot tested on different strains of *S.*

TABLE 1. Bacterial strains

Strain	R-M phenotype	Relevant genotype/phenotype	Source or reference
S. typhimurium LT2			
4247	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA22 metE551 trpD2 strA120*	SL1027 of Stocker et al. (8)[a]
4274	$r_{LT}^- m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA22 metE551 trpD2 ilv452 hsdLT6 strA120*[b]	2
4278	$r_{LT}^- m_{LT}^- r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA22 metE551 trpD2 ilv452 hsdLT6 strA120*[b]	2
4419	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+$	*metA22 metE551 trpD2 ilv452 hsdLT6 hsdSA29 strA120*[b]	2, 7
4529	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*Δchl(gal bio uvrB)1013* F′ 6-*gal*$^+$	SL1694 of B. A. D. Stocker (personal communication)
S. typhimurium LT2/*S. potsdam* hybrid			
3003	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SP}^+ m_{SP}^+$	*metA22 metE551 trpD2 ilv-452 hsdSB* (*pyrB*$^+$ *hsdSP*$^+$) *strA120*[c]	4
E. coli K-12/*S. typhimurium* LT2 hybrids			
4617	$r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	*thi lac* (*serB80 leu*$^+$)[d]	2
4619	$r_{SA}^+ m_{SA}^+ r_{SB}^- m_{SB}^-$	*thi lac hsdSB* (*serB80 leu*$^+$)[d]	9
E. coli C			
JR3		F Tet^r	P1 transduction from JW407 from J. Wechsler to F101; transfer to *E. coli* C

[a] A more complete genotype of strain SL1027 is given in reference 8; genes omitted here are irrelevant to the present study.

[b] In reference 7, only the genotype of strain 4419 (=CL4419) is given. Since both strains 4274 and 4278 are in the ancestry of strain 4419, those genes of strains 4274 and 4278 in common with strain 4419 have been given identical designations.

[c] Genes in parentheses are from *S. potsdam*. Since the recipient parent of strain 3003 was a derivative of strain 4419 (2), the common genes have identical designations.

[d] Genes in parentheses are from *S. typhimurium* LT2.

TABLE 2. Efficiency of plating of different phages with various modifications on strains of *S. typhimurium*[a]

Strain	R-M phenotype	Efficiency of plating of phage:[b]					
		P22. LT, SA, SB	P22. SA, SB	L. LT, SA, SB	L. LT, SB	P3. LT, SA, SB	P3. LT, SA, SP[c]
4247	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	1.0	10^{-4}	1.0	10^{-2}	1.0	10^{-3}
4274	$r_{LT}^- m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	1.0	1.0	1.0	10^{-2}	1.0	10^{-3}
4419	$r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+$	1.0	1.0	1.0	1.0	1.0	10^{-3}
4278	$r_{LT}^- m_{LT}^- r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	1.0	1.0	1.0	10^{-1}	1.0	10^{-3}
LB5000		1.0	1.0	1.0	1.0	1.0	1.0

[a] Dilutions (100-fold) of phage lysates with titers between 10^8 and 10^9 PFU/ml were spotted onto lawns of the test bacteria (grown overnight at 30°C), allowed to dry in, and incubated overnight at 37°C, and the efficiency of plating was determined (3).

[b] Phage notations follow the system previously used in which the letters after the phage designation indicate the phage modifications (3).

[c] SP indicates SP modification (2); the significance of using phage with this modification is merely that it lacks SB modification. SB and SP are allelic (2).

TABLE 3. Percentages of Tet^r colonies derived from *S. typhimurium* strains 4247, 4274, 4419, and LB5000 after infection with the F Tet^r plasmid from *E. coli* C JR3[a]

Strain	Restriction phenotype	% Tet^r colonies		
		30°C	37°C	42°C
4247	$r_{LT}^+ r_{SA}^+ r_{SB}^+$	$<1 \times 10^{-6}$	4.0×10^{-5}	8.2×10^{-4}
4274	$r_{LT}^- r_{SA}^+ r_{SB}^+$	4.8×10^{-4}	2.1×10^{-3}	1.4×10^{-2}
4419	$r_{LT}^- r_{SA}^- r_{SB}^+$	9.8×10^{-3}	8.5×10^{-2}	1.2×10^{-1}
LB5000	$r_{LT}^- r_{SA}^- r_{SB}^-$	5.6×10^{-1}	13	12

[a] Exponential-phase cells of Str^r *S. typhimurium* recipient strains 4247, 4274, 4419, and LB5000 were all grown at 30, 37, and 42°C, mixed with exponential-phase cells of Str^s *E. coli* C JR3 at a donor-to-recipient cell ratio of 1:10, incubated without shaking at the same temperature for 1 h, and plated out on L agar containing 100 μg of streptomycin plus 10 μg of tetracycline per ml. Plates were incubated overnight at the respective temperatures. The numbers in the table are the frequencies of Tet^r colonies derived from each *S. typhimurium* recipient, expressed as a percentage of the number of *S. typhimurium* cells in the mixture.

typhimurium LT2 and *E. coli* K-12 with known R-M phenotypes, and their efficiencies of plating were determined. Since each of the phages plated with an efficiency of plating of 1.0 on all their sensitive strains, LB5000 had the modification phenotype $m_{LT}^+ m_{SA}^+ m_{SB}^+$. Thus, the complete R-M phenotype of LB5000 is $r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^- m_{SB}^+$. Additionally, LB5000 acquired a requirement for leucine. Thus, with respect to its nutritional requirements, the genotype of LB5000 is *metA22 metE551 trpD2 leu*.

To determine whether LB5000 was an efficient recipient of unmodified DNA, we compared the numbers of Tet^r colonies formed by each of the *S. typhimurium* strains 4247, 4274, 4419, and LB5000 after transfer of the F Tet^r plasmid from *E. coli* C JR3. Since *E. coli* C is a naturally occurring $r^- m^-$ isolate of *E. coli*, plasmid DNA propagated in this organism is unmodified. The numbers of Tet^r colonies expressed as percentages of the numbers of Tet^s recipient cells in the donor-recipient cell mix are shown in Table 3. The progressive increase in the frequency of Tet^r colonies with strain 4247, which possessed all three restrictions, through to the triple restrictionless mutant LB5000 indicated a progressive loss of restriction with each new restriction mutation. Thus, LB5000 was an efficient recipient of unmodified DNA. The increase in the frequencies of Tet^r colonies with increased temperatures, also seen from the results in Table 3, was a reflection of the temperature sensitivity of the *S. typhimurium* restrictions (5). Additionally, the results indicated that although the DNAs of different phages manifested different sensitivities to the three *S. typhimurium* restrictions, unmodified DNA from other sources could be expected to be sensitive to all three restrictions.

Transduction from *E. coli* to *S. typhimurium* can be achieved with phage P1. Ornellas and Stocker (7) showed that *galE* mutants of *S. typhimurium* LT2 were P1^s, and Bullas et al. (3) showed that P1^s strains of different *Salmonella* serotypes could be selected by their resistance to phage FO. Accordingly, we isolated spontaneous *galE* mutants of LB5000 by examining FO^r colonies and determining their sensitivity to phage P1. In this way, we isolated a *galE* P1^s strain of LB5000 with the same auxotrophic requirements as LB5000, which we designated LB5010. Sensitivity to phages P22, L, and P3 was restored by infection with F *gal*$^+$ from *S. typhimurium* 4529 (3). When this was done, the R-M phenotype of LB5010 was the same as that of LB5000.

Cultures of LB5000 and LB5010 are also available from the Salmonella Genetic Stock Center, Department of Biology, University of Calgary, Calgary, Canada T2N 1N4.

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