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 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 rSB− mSB+ mutant of 4419 after additional treatment with N-methyl-N'-nitro-N-nitrosoguanidine (NG) (4).

We screened for rSB− 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 rSB+ cells but not by rSB− cells. Thus rSB− 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 rSB− by using phage P3. These mutants were further examined with phages P22 and L to verify that they were still rLT− and rSA−. 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 fLT− fSA− rSB−.

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.
E. coli

\( (2) \), designations.

phage modifications

the

rLT4278

SB

modification.

plating was

4247

rLT\(^+\) mL\(^+\) rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \text{metA22 metE551 trpD2} \)

strA120

SL1027 of Stocker et al. (8)\(^a\)

4274

rLT\(^-\) mL\(^+\) rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \text{metA22 metE551 trpD2} \)

ilv432 hsdLT6 strA120\(^b\)

2

4278

rLT\(^-\) mL\(^+\) rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \text{metA22 metE551 trpD2} \)

ilv432 hsdLT6 strA120\(^b\)

2

4419

rLT\(^-\) mL\(^+\) rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \text{metA22 metE551 trpD2} \)

ilv452 hsdLT6 hsdSA29 strA120\(^b\)

2, 7

4529

rLT\(^+\) mL\(^+\) rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \Delta \chi(k\text(gal bio uvrB)}1013 F^+ \)

6-gal\(^+\)

SL1694 of B. A. D. Stocker (personal communication)

S. typhimurium LT2/S. potsdam hybrid

3003

rLT\(^+\) mL\(^+\) rSA\(^+\) mSA\(^+\) rSP\(^+\) mSP\(^+\)

\( \text{metA22 metE551 trpD2} \)

ilv452 hsdSB (pyrB\(^+\) hsdSP\(^+\) ) strA120\(^b\)

4

E. coli K-12/S. typhi-

murium LT2 hybrids

4617

rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \text{thi lac (serB80 leu)}^+\)

2

4619

rSA\(^+\) mSA\(^+\) rSB\(^+\) mSB\(^+\)

\( \text{thi lac hsdSB (serB80 leu)}^+\)

9

E. coli C

JR3

F Tet\(^+\)

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\(^a\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>R-M phenotype</th>
<th>Efficiency of plating of phage: (^b)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LT, SA, SB</td>
<td>SA, SB</td>
</tr>
<tr>
<td>4247</td>
<td>rLT(^+) mL(^+) rSA(^+) mSA(^+) rSB(^+) mSB(^+)</td>
<td>1.0</td>
</tr>
<tr>
<td>4274</td>
<td>rLT(^-) mL(^+) rSA(^+) mSA(^+) rSB(^+) mSB(^+)</td>
<td>1.0</td>
</tr>
<tr>
<td>4419</td>
<td>rLT(^-) mL(^+) rSA(^+) mSA(^+) rSB(^+) mSB(^+)</td>
<td>1.0</td>
</tr>
<tr>
<td>4529</td>
<td>rLT(^+) mL(^+) rSA(^+) mSA(^+) rSB(^+) mSB(^+)</td>
<td>1.0</td>
</tr>
<tr>
<td>LB5000</td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^a\) 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\(^\circ\)C), allowed to dry in, and incubated overnight at 37\(^\circ\)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).
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 $r_{LT}^+ r_{SA}^+ r_{SB}^+$. 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' colonies formed by each of the S. typhimurium strains 4247, 4274, 4419, and LB5000 after transfer of the F Tet' 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' colonies expressed as percentages of the numbers of Tet' recipient cells in the donor-recipient cell mix are shown in Table 3. The progressive increase in the frequency of Tet' 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' 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 PI', and Bullas et al. (3) showed that PI' strains of different Salmonella serotypes could be selected by their resistance to phase FO. Accordingly, we isolated spontaneous galE mutants of LB5000 by examining FO' colonies and determining their sensitivity to phase PI. In this way, we isolated a galE PI' strain of LB5000 with the same auxotrophic requirements as LB5000, which we designated LB5010. Sensitivity to phases 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.

The technical assistance of Leone Merriam is greatly appreciated. The encouragement of Ken Sanderson to isolate the strains which are the subject of this paper is also acknowledged and appreciated.

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LITERATURE CITED


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### Table 3. Percentages of Tet' colonies derived from S. typhimurium strains 4247, 4274, 4419, and LB5000 after infection with the F Tet' plasmid from E. coli C JR3*"

<table>
<thead>
<tr>
<th>Strain</th>
<th>Restriction phenotype</th>
<th>% Tet' colonies at 30°C</th>
<th>% Tet' colonies at 37°C</th>
<th>% Tet' colonies at 42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4247</td>
<td>$r_{LT}^+ r_{SA}^+ r_{SB}^+$</td>
<td>$&lt; 1 \times 10^{-6}$</td>
<td>$4.0 \times 10^{-3}$</td>
<td>$8.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>4274</td>
<td>$r_{LT}^- r_{SA}^- r_{SB}^+$</td>
<td>$4.8 \times 10^{-4}$</td>
<td>$2.1 \times 10^{-3}$</td>
<td>$1.4 \times 10^{-2}$</td>
</tr>
<tr>
<td>4419</td>
<td>$r_{LT}^- r_{SA}^+ r_{SB}^+$</td>
<td>$9.8 \times 10^{-3}$</td>
<td>$8.5 \times 10^{-2}$</td>
<td>$1.2 \times 10^{-1}$</td>
</tr>
<tr>
<td>LB5000</td>
<td>$r_{LT}^- r_{SA}^- r_{SB}^+$</td>
<td>$5.6 \times 10^{-1}$</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

* Exponential-phase cells of Str' 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' 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' colonies derived from each S. typhimurium recipient, expressed as a percentage of the number of S. typhimurium cells in the mixture.

