Fertility of *Salmonella typhimurium* Crosses with *Escherichia coli*

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At least one factor that causes low fertility of *Salmonella typhimurium* LT2 strains in crosses with *Escherichia coli* K-12 Hfr's can be inhibited by growing the female strains in supplemented minimal salts medium rather than in nutrient broth and by incubating the female strains at 50°C immediately before mating with the Hfr. These pretreatments can enhance the recovery of prototrophic recombinants for markers injected early by the Hfr by a factor of as much as 10⁴. The heat treatment is effective only on the female in intergeneric crosses and gradually loses (within 50 min) its effectiveness after return of heat-treated cells to 37°C. It is concluded that the restriction system of the female is heat-sensitive. Since markers injected late by the male enter females in which the heat-impaired restriction system has recovered, few recombinants for late markers are found. The presence of the leading end of an *E. coli* Hfr in an *S. typhimurium-E. coli* hybrid enhances by up to sevenfold the frequency of lac⁺ recombinants in subsequent crosses with an *E. coli* Hfr if the *E. coli* segment is integrated into the chromosome of the hybrid; the effect is less marked if the *E. coli* segment is not integrated.

The fertility of *Salmonella typhimurium* strains in conjugal crosses with *Escherichia coli* male strains is very low (1, 27). There are at least three factors responsible for the low fertility of these intergeneric crosses (Middleton and Mojica-a, Advan. Genet. in press): differences in cell surface, effects of female restriction on male deoxyribonucleic acid (DNA), and differences in DNA sequences. Low fertility has been overcome by isolating fertile (fer) mutants which yield more prototrophic hybrids than do fer⁻ female strains (1, 17). The impaired restriction system of fer mutants (18) makes difficult the genetic analysis of some hybrids by transduction. *S. typhimurium* LT7 strains which carry a mutator (mut) gene also behave as fertile females (17). *S. typhimurium* LT2 strains are fertile (10) in the presence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) which may, however, have unselected genetic consequences. This report describes the markedly increased fertility of *S. typhimurium* females as a result of experimental manipulation of the female restriction mechanism. The temporary enhancement of female fertility is in agreement with the comparative study of restriction in *S. typhimurium* (6, 18) and avoids the use of mutagens (10). In addition, the increased fertility of female strains which carry male genetic material homologous with the leading end of the Hfr chromosome (15) is confirmed.

**MATERIALS AND METHODS**

**Bacterial strains.** Bacterial strains used are described in Table 1. All *S. typhimurium* strains are derived from LT2, since most genetics and biochemistry have been done with derivatives of LT2 (20), and have an SQ prefix (assigned to R.B.M. by K. E. Sanderson for the *Salmonella* Information Exchange) in their strain numbers. All *E. coli* strains are derivatives of K-12. The hybrid recombinants discussed in this report are *S. typhimurium-E. coli* Trp⁺ recombinants and are designated by the TC10 prefix. The nomenclature is that of Demerec et al. (9).

**Phage strains.** Phage H5, virulent mutant of *S. typhimurium* phage P22, is from the stock collection of R.B. Middleton. Coliphage P1-virulent was kindly sent by A.L. Taylor.

**Media.** The minimal salts medium (MM) that of Sanderson and Demerec (21); L-amino acids were added when required to a final concentration of 20 mg/liter. Minimal agar (MA) was MM plus 1.5% agar (Difco). Single enriched minimal agar (SEMA) was MA plus 1.25% (v/v) Difco nutrient broth. Difco nutrient broth (NB) and Difco nutrient agar (NA) were prepared according to the specifications of the manufacturer except that 0.5% NaCl was added. Slant agar was double strength NA.

**General methods.** Strains were kept in agar slants, stored at 4°C, and transferred about every 10 weeks. Before use each strain was grown overnight in NB and streaked twice for single-cell isolation on selective
plates. Strains were tested for sensitivity to P1-virulent and H5 phages by cross-streaking dilute cell suspensions on NA. All incubations were at 37 C.

**Conjugation techniques.** Female refers to a strain used in a conjugation cross with a male (Hfr). All male strains were grown in NB, without aeration, to late log phase (8). All female strains were grown to saturation with vigorous aeration. For heat treatment, small volumes of cells were placed in preheated tubes at 50 C for 20 to 40 min immediately before mating. Matings were performed by one of two methods which yielded essentially identical results: (i) portions of male and female cells were plated on selective plates (usually SEMA) or (ii) after incubation in wide-bottom flasks for desired times, matings were interrupted by shaking on a Vortex shaker for 2 min and 0.1-ml portions were spread on selective plates. Before mating females were grown as follows: (i) in NB, (ii) in MM supplemented with required amino acids, (iii) in NB and heated, or (iv) in supplemented MM and heated. Hybrid recombinants were counted after 72 hr of incubation.

**Immunospecificity.** *E. coli* antiserum polyvalent B was purchased from BBL division of Bioquest, Cockeysville, Md., and was used undiluted. *S. typhimurium* antiserum H (prepared against a mixture of phases 1 and 2) and O were gifts of S. I. Vas, Department of Microbiology and Immunology, McGill University. Antiserum H was diluted 1:200 and antiserum O was diluted 1:50. Recombinants were tested as described by St. Pierre and Demerec (19).

### RESULTS

**Antigen studies and phage sensitivity.** In crosses in which the number of recombinants was less than 100, all recombinants were tested; when over 100 recombinants resulted, 100 randomly selected recombinants were tested. All recombinants gave a positive reaction with both *S. typhimurium* antisera, and none gave a positive reaction with *E. coli* antiserum. None of the recombinants tested was sensitive to coliphage P1 and most were sensitive to phage H5. These tests distinguished between reversion of the male parent (counterselected markers were point mutations) and recombinant formation (most female parents carried deletion markers). The basis for the lack of phage H5-sensitivity of some recombinants is not known, but there appears to exist a correlation of low or high proportion of H5-sensitive recombinants with particular selected markers in the females.

**Stability.** Hybrids from interfamilistic matings are often unstable heterogenotes (2) which characteristically revert to auxotrophy characteristic of the female parent after passage through single-cell isolation; a varying proportion of hybrids do remain stable, however (Table 2).

**Recombinant yield.** In conventional crosses the yield of recombinants between *S. typhimurium* LT2 females and *E. coli* males is of the order of 10^-7~10^-8 (reference 10; Table 2, column 1) many of which are lost upon single-cell isolation. The growth of females in supplemented MM rather than in NB results in approximately a 20-fold increase in the yield of recombinants (Table 2, column 2). In addition, a larger proportion of hybrids from MM-grown females are stable than from NB-grown females. Preincubating NB-grown females at 50 C for 20 min (Table 2, column 3) results in approximately a 200-fold increase in hybrid recombinants over females not incubated at 50 C. Table 2, column 4 shows that by selecting for Trp^- recombinants from Hfr B7.

### Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Characteristics</th>
<th>Source or (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhimurium</em> LT2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQ45</td>
<td>Wild type</td>
<td>R. Middleton</td>
</tr>
<tr>
<td>525</td>
<td>trpA512 deletion</td>
<td>(5)</td>
</tr>
<tr>
<td>518</td>
<td>trpB223 point mutation</td>
<td>(5)</td>
</tr>
<tr>
<td>521</td>
<td>trpE95 deletion</td>
<td>(5)</td>
</tr>
<tr>
<td>711</td>
<td>trpE141 deletion</td>
<td>(5)</td>
</tr>
<tr>
<td>519</td>
<td>trpD9 point mutation</td>
<td>(5)</td>
</tr>
<tr>
<td>712</td>
<td>trpD119 deletion</td>
<td>P. McCann</td>
</tr>
<tr>
<td>526</td>
<td>trpC109 deletion</td>
<td>(5)</td>
</tr>
<tr>
<td>713</td>
<td>trpA8E30</td>
<td>(5)</td>
</tr>
<tr>
<td>714</td>
<td>trpB8ED63</td>
<td>(3)</td>
</tr>
<tr>
<td>715</td>
<td>trpA8EDC167</td>
<td>(5)</td>
</tr>
<tr>
<td>716</td>
<td>supX34 deletion</td>
<td>(16)</td>
</tr>
<tr>
<td>717</td>
<td>trpA8EDC101 cysB259</td>
<td>(16)</td>
</tr>
<tr>
<td>413</td>
<td>hisF41 deletion</td>
<td>R. Middleton</td>
</tr>
<tr>
<td>516</td>
<td>pro215</td>
<td>R. Middleton</td>
</tr>
<tr>
<td>372</td>
<td>cysC1021</td>
<td>R. Middleton</td>
</tr>
<tr>
<td>718</td>
<td>Hfr H5 thi O-cysB trp his argB hemA-F</td>
<td>(20)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A905</td>
<td>trpA905 deletion</td>
<td>C. Yanofsky</td>
</tr>
<tr>
<td>AE1</td>
<td>trpABCDE1</td>
<td>(26)</td>
</tr>
<tr>
<td>BS1</td>
<td>trpABCDE cysB</td>
<td>R. Somerville</td>
</tr>
<tr>
<td>B2</td>
<td>trpB2 point mutation</td>
<td>(14)</td>
</tr>
<tr>
<td>B7</td>
<td>Hfr B7 metO-trp pyrD</td>
<td>(25)</td>
</tr>
<tr>
<td>purF lac...pabB-F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB311</td>
<td>Hfr AB311 thi O-his pyrF trp bioA...pur-F</td>
<td>(25)</td>
</tr>
<tr>
<td>P4X6</td>
<td>Hfr P4X6 metB1 O-proB proA leu...lac-F</td>
<td>(25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><em>S. typhimurium</em> ×</th>
<th><em>E. coli</em> Trp^- hybrids</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TC10-21</td>
<td>SQ525 × B7 Stable</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>SQ525 × B7 Unstable*</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>SQ526 × B7 Stable</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>SQ526 × B7 Unstable</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>SQ715 × B7 Stable</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>SQ715 × B7 Unstable</td>
<td></td>
</tr>
</tbody>
</table>

* Unstable hybrids segregate Trp^- clones and carry a cryptic trp gene.
(injects the *trp* region very early) and from females grown on supplemented MM and incubated at 50 °C for 20 min, the overall frequency of *Trp*" recovery is 2.28 × 10^-4, a more than 2 × 10^4 enhancement of recombination over conventionally prepared females (37 °C in NB). This increased frequency in recombination is similar to that obtained with *fer* females.

A few strains (e.g., SQ519) fail to give stable recombinants and others (SQ521, SQ712) fail to give stable His-sensitive prototrophic recombinants (not shown in Table 2). Large *trp* deletions (SQ714, SQ713, SQ715) tend to give higher yields of prototrophic recombinants than do shorter deletions or point mutations. Table 2 shows that heat treatment does not increase the frequency of recombination for markers which are injected late by the male to the same extent as for markers injected early. For example, *trp*" genes are injected after about 70 min by Hfr P4X6, and the frequency of *Trp*" recombinants is much lower than the frequency of Pro" recombinants (injected early by Hfr P4X6). In contrast, Trp" recombinants are markedly more frequent than Pro" recombinants when the male is Hfr B7 (injects *trp* early and Pro" after about

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**Table 2. Effects of pretreatments of the females on the frequency of recombinants in intergeneric crosses**

<table>
<thead>
<tr>
<th>Females</th>
<th>Pretreatment conditions for females</th>
<th>Percentage of recombinants before treatment</th>
<th>Average frequency for early markers</th>
<th>Percentage of recombinants after treatment</th>
<th>Average frequency for early markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQ525 B7 (trp&quot; injected early)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQ525 P4X6 (trp&quot; injected late, pro+ injected early)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQ525 AB311 (trp&quot; injected late, his+ injected early)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- All females are *trp* except as indicated.
- Frequencies expressed as × 10^-4.
- Percentage of hybrid recombinants that do not revert to auxotrophy after single-cell isolation: --, not tested.
- Frequencies for markers injected late are given in parentheses.
A similar observation was made with Hfr AB311, which injects his+ very early. Heat treatment of the male strain (results not shown) does not increase the recovery of recombinants.

These observations can be interpreted in two ways: the lack of homology between the female and male chromosomes limits the extent of transfer as predicted by current conjugation models (7), and the effect on the female of heat pretreatment is temporary and does not last long enough to enhance recombination for distal male markers. The possibility that the recombination-enhancing effect of heat treatment is temporary was explored. Table 3 and Fig. 1 describe the frequencies of Trp+ recombinants in crosses where the female has been pretreated and mated with the male at different times after pretreatment. The effect of heat treatment is temporary and can no longer be detected after 50 min when the yield of recombinants is approximately equal to the yield from untreated females (Table 2, column 2).

If the primary effect of heat pretreatment is on the restriction system of the female, three properties would be expected: in addition to a most pronounced effect for early male markers as already shown (Table 2) and disappearance of the effect after return of the female to 37 C (Table 3; Fig. 1), there would only be an effect when the female strain could restrict male DNA, e.g., in intergeneric crosses already mentioned. There should be no effect of heat pretreatment on intraspecific crosses (S. typhimurium Hfr × S. typhimurium females and E. coli Hfr × E. coli females). Table 4 shows the results of intraspecific crosses in which the females have undergone various treatments (columns 1–4). Column 5 shows heat pretreatment of the males, and column 6 shows the results when both males and females were pretreated at 50 C. Each female yields a characteristic frequency of recombinants regardless of the various treatments. The results in Table 4 show that incubation at 50 C before mating is not effective in increasing the yield of recombinants in intraspecific crosses.

Homologous leading end of E. coli Hfr. S. typhimurium Trp+ hybrids selected in matings with E. coli Hfr B7 possess a male genetic segment corresponding to the earliest injected region of the male. Those which are unable to utilize lactose as a carbon source can be remated with E. coli Hfr B7 in order to observe the effect of the presence of the Hfr leading end in a hybrid on recombination frequency for other male markers (15). Hfr B7 injects the lac+ genes after about 15 min. Table 5 shows that the presence in hybrids of the leading end of the E. coli Hfr has a detect-
able enhancing effect (up to sevenfold) on the yield of lac+ recombinants. This effect is not expressed when the hybrid is extremely unstable (TC10-26) and only partially expressed when the hybrids are somewhat unstable (TC10-43 and -56). The basis for partial enhancement in unstable recombinants is not clear, but there is no impairment of the restriction machinery of the hybrids which are able to plate phage P22 as efficiently as wild-type LT2 (Mojica-a and Middleton, in preparation). A further indication that the restriction mechanism is unimpaired is the effectiveness of heat treatment in further enhancing the frequency of lac+ recombinants (Table 5). It also appears (Table 5) that a longer piece of male DNA in the hybrid is more effective in increasing the frequency of lac+ recombinants. Hybrids (TC10-21 and -55) in which only one trp+ male gene was selected show a three- to fourfold increase. A hybrid (TC10-45) with five trp+ male genes shows a sevenfold increase in lac+ recombinants as compared with its trp female parent (SQ715).

DISCUSSION

The term fertility here describes the ability of S. typhimurium strains to act as females in conjugation with E. coli males. Increases in fertility from changing the growth medium (Table 2, column 2) and from elevating the preincubation temperature (Table 2, columns 3 and 4) are consistent with alterations of the restriction mechanism (11-13, 23, 24) of the female. The effect of heat treatment declines after return of the heat-treated females to 37 C and disappears after 50 min (Table 3; Fig. 1). The incubation of the females at 50 C for 20 to 40 min does not affect viability, which begins to decline if incubations continue longer than 50 min, but rather affects either the synthesis or the activity of the restriction enzymes which recover full activity after 50 min. The fertility-enhancing effect of the heat treatment is specific for the female in intergeneric crosses and has no effect on the female in intraspecific crosses (Table 4) where restriction would presumably not be operating. In addition, although the presence of E. coli genetic material has an enhancing effect on the frequency of recombination for additional male genes (Table 5), hybrid recombinants still have a thermosensitive fertility mechanism as is expected if the heat treatment acts on the female and the effect is temporary. Ultraviolet irradiation (UV) also has an enhancing effect on the frequency of recombination formation (Mojica-a, unpublished data) in agreement with the demonstration that restric-

**TABLE 4. Frequencies of trp+ recombinants in intraspecific crosses**

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Males* preincubated at 37 C</th>
<th>Males* preincubated at 50 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NB* 37 C</td>
<td>MM + TRP* 37 C</td>
</tr>
<tr>
<td>* Males were grown in nutrient broth.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Female growth and preincubation conditions. NB, nutrient broth; MM, minimal salts medium; TRP, tryptophan.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Frequencies expressed as x 10^-3.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The fertility of *S. typhimurium* LT2 strains can be increased by preincubation at 50 °C without possibly uncontrolled genetic effects of UV or NG or changed host specificity of transducing phage prepared on hybrid recombinants from a *fer* parent. This new protocol makes accessible for the routine production of hybrids the numerous, genetically well characterized *S. typhimurium* LT2 strains. The effects of the protocol are generalized over the chromosome as shown by increased recombination frequencies for male *trp*, *his*, *pro*, and *lac* genes (Table 2 and 5). For optimum results, an Hfr strain that injects the selected genes early must be used (Table 2). Within a region of the chromosome, however, there are additional specific effects of the female markers on the frequency of recombination; for example, of two *trpD* mutants used (SQ519, SQ715) one gives twice as many recombinants as the other, and of two *trpE* mutants (SQ521, SQ711) one gives almost 10 times as many recombinants as the other (Table 2). Long deletions tend to give higher recombination frequencies than short deletions or point mutations (cf. SQ518 and SQ715 in Table 2). Whether this is due to the inversion of the *trp* region (22) in the two species cannot be determined from the present data.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


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**TABLE 5. Frequency of lac* recombinants**

<table>
<thead>
<tr>
<th>Females</th>
<th>NB&lt;sup&gt;a&lt;/sup&gt; 37 C</th>
<th>MM + TRP&lt;sup&gt;b&lt;/sup&gt; 37 C</th>
<th>NB&lt;sup&gt;c&lt;/sup&gt; 50 C, 20 min</th>
<th>MM + TRP&lt;sup&gt;c&lt;/sup&gt; 50 C, 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhimurium trp</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQ421</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>0.6</td>
<td>5.9</td>
</tr>
<tr>
<td>525</td>
<td>0.1</td>
<td>0.1</td>
<td>1.1</td>
<td>7.1</td>
</tr>
<tr>
<td>526</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>1.2</td>
<td>6.7</td>
</tr>
<tr>
<td>715</td>
<td>&lt;0.1</td>
<td>0.3</td>
<td>0.9</td>
<td>9.2</td>
</tr>
</tbody>
</table>

*trp<sup>c</sup> Hybrids (from trp females) |
| TCV-21 (SQ525) | 0.3 | 0.9 | 4.5 | 23.6 |
| 26<sup>c</sup> (SQ525) | <0.1 | 0.1 | 0.7 | 4.8 |
| 55 (SQ526) | 0.1 | 0.2 | 3.1 | 31.1 |
| 56<sup>c</sup> (SQ526) | <0.1 | 0.3 | 2.3 | 12.5 |
| 45 (SQ715) | 0.6 | 2.4 | 11.7 | 65.4 |
| 43<sup>c</sup> (SQ715) | 0.2 | 0.7 | 3.6 | 29.9 |

<sup>a</sup>Lac<sup>+ </sup>recombinants were scored on minimal agar in which lactose (0.5% w/v) was substituted for glucose. Frequencies are expressed as × 10<sup>-6</sup>. Note that hybrids with an integrated leading end (trp<sup>c</sup>) of *E. coli* Hfr B7 yield more recombinants for a later male marker (lac<sup>-</sup>) in matings with the *E. coli* male than do *S. typhimurium trp* females.

<sup>b</sup>Female growth and preincubation conditions. NB, nutrient broth; MM, minimal salts medium; TRP, tryptophan.

<sup>c</sup>Unstable hybrids (TC10-26, -56, -43) are less effective in increasing the frequency of lac<sup>+</sup> recombinants than are stable hybrids (TC10-21, -55, -45).
FERTILITY OF INTERGENERIC CROSSES

Genetics 47:1043-1052.


