Mobilization of Non-conjugative Tetracycline, Streptomycin, Spectinomycin and Sulphonamide Resistance Determinants of Escherichia coli

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SUMMARY

Non-conjugative Tc, Sm, SmSu, SmSpc, SmSpcSu and TcSmSpcSu determinants (Tc, Sm, Spc and Su denote resistance to tetracycline, streptomycin, spectinomycin and sulphonamides, respectively) in wild-type Escherichia coli strains were mobilized with transfer factors F, I and A2 and implanted in E. coli K12. F and I were transmitted at very high rates in all matings in which these E. coli K12 strains were used as donors; the rate of A2 transmission was not measured. When the Tc determinants, derived from 32 of the wild-type E. coli strains, were serially transferred between strains of E. coli K12, they were transmitted either at a very low rate in both first and second matings, at a very low rate in the first mating and at a very high rate in the second mating, or at a very high rate in both matings. In the donors that transmitted at the low rate, the Tc determinants were probably chromosomally located, a recombination event between them and the transfer factors being responsible for the formation of the donors that transmitted at the high rate; once they became extrachromosomally located, the Tc determinants continued to be transmitted at the high rate. Of the resistance determinants studied, Tc was the only one suspected of being chromosomally located.

F transmitted most of the four Sm and eight SmSpc determinants at high rates and the 14 SmSu determinants at much lower rates. I and A2 transmitted most of the three kinds of determinants at high rates but four of the E. coli K12 SmSpc+A2+ strains transmitted them at very low rates and two E. coli K12 SmSpc+I+ strains and one E. coli K12 SmSu+I+ strain did not transmit them at all. F and I did not establish linkage with Sm or SmSu but linkage was detected in the strains that transmitted at the high rate between them and SmSpc. The one TcSmSpcSu determinant studied was transmitted at two moderately low rates by F with which it was not linked. In the first matings following implantation in E. coli K12 by I or A2, it was transmitted at a very low rate, but in most subsequent matings its transmission rate was high; for the I+ strain this was certainly due to transfer factor linkage being established.

INTRODUCTION

As a result of routinely examining enterobacteria in this laboratory for transmissible antibiotic resistance, a collection of Escherichia coli strains had accrued whose tetracycline, streptomycin, spectinomycin or sulphonamide resistance could only be transmitted to other strains if transfer factors were implanted in them. Because the strains came from a variety of sources and, as far as could be determined, were epidemiologically unrelated, they presented an opportunity of studying, in the same bacterial host, a wide selection of genetic elements determining the same kind of antibiotic resistance. This paper deals with their location in the common host, the rate at which they were transferred by different transfer factors and the relationship they established with these transfer factors.
METHODS

Strains of E. coli. All the wild-type strains possessing non-conjugative determinants for resistance to tetracycline (Tc), streptomycin (Sm), spectinomycin (Spc) or sulphonamides (Su) had been isolated from the faeces of healthy humans, calves, pigs or domestic fowl; they had been tested several times by the method of Smith & Gyles (1970) before their resistance had been recorded as non-transmissible. Two E. coli K12 strains differing in several easily identified characteristics (Smith & Gyles, 1970) were used in most conjugation experiments. One, strain Proto, was an amp' lac+ prototroph and the other, strain Auxo, was a nal' lac auxotroph requiring histidine, phenylalanine, proline and tryptophan. Stationary nutrient broth cultures (Oxoid no. 2) incubated for 24 h at 37 °C were used throughout.

The mobilization of non-conjugative resistance determinants by transfer factors. This was attempted with three transfer factors, F (Lederberg, Cavalli & Lederberg, 1952; Hayes, 1953), I (Idrd16; Hardy et al., 1973) and A2 (Smith & Heller, 1973), in different Auxo strains by the method of Anderson (1965) using techniques described by Smith & Gyles (1970) and Smith & Linggood (1970). Strain Auxo was also used as the final recipient of those determinants that were mobilized.

Transfer of antibiotic resistance between E. coli K12 strains. Nutrient broth was inoculated with 0.02 ml each of broth cultures of the Proto strain and of the Auxo strain in which antibiotic resistance determinants and a transfer factor had been implanted in the mobilization experiments. After incubation, the proportion of organisms of strain Proto in the mixed culture that had acquired these determinants from the Auxo strain (referred to as the transfer rate) was estimated from viable counts (Miles & Misra, 1938) on agar media containing ampicillin alone, and ampicillin and each of the antibiotics against which the determinants provided resistance. (When re-transferring determinants from strain Proto to strain Auxo the procedure was the same except that the ampicillin in the selection medium was replaced by sodium nalidixate). Ten colonies from each of the plates that contained two antibiotics were examined to see whether they had acquired from the donor strain all its resistance determinants and, in the case of F and I matings, its transfer factor; one colony was purified, checked for Auxo or Proto nutritional character when the transfer rate was low and retained for use in subsequent matings. Acquisition of F or I was estimated by testing 16 colonies that grew on the ampicillin-containing medium for susceptibility to visual lysis by the F-specific phage MS2 (Davis, Strauss & Sinsheimer, 1961) or the I-specific phage If1 (Meynell & Lawn, 1968). Some E. coli K12 strains in which determinants had been implanted with transfer factor A2 were visually lysed by the If1 phage, but the reaction was often not sufficiently clear-cut to make the test routinely applicable for identifying A2+ strains. Sulphonamide-resistant organisms were estimated on Sensitest Agar (Oxoid, CM409) and other resistant organisms on MacConkey agar. The concentrations of sulphonamide (sulphafurazole) and streptomycin in the medium were 75 and 7.5 μg ml⁻¹ respectively; the concentration of other antibiotics used was 20 μg ml⁻¹.

Loss of resistance determinants. This was assessed after growth at 37 °C in broth containing sodium lauryl sulphate (Tomoeda et al., 1968); five serial transfers from small inocula were made before each strain was examined for loss of determinants.

Antibiotic sensitivity tests. These were done on Sensitest Agar by the disc method (Smith, 1970) using an Oxoid Multodisk (1744E) (containing (μg): oxytetracycline, 50; chloramphenicol, 50; neomycin, 30; nalidixic acid, 30; streptomycin, 25; ampicillin, 25; furazolidone,
Table 1. Mobilization of antibiotic resistance determinants in wild-type strains of E. coli by different transfer factors

The determinants in all the strains were mobilized by one or other of the transfer factors.

<table>
<thead>
<tr>
<th>Determinants</th>
<th>F</th>
<th>I</th>
<th>A2</th>
<th>F, I or A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc</td>
<td>22</td>
<td>27</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Sm</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>SmSu</td>
<td>11</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>SmSpc</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>SmSpcSu</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TcSmSpcSu</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Any of the above</td>
<td>42</td>
<td>48</td>
<td>54</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 2. Transfer rates of the Tc determinants of 32 wild-type E. coli strains between strains of E. coli K12

In all matings which they mediated, F and I transferred at rates of $10^6$ to $10^{-1}$; the rate of A2 transfer was not assessed. In each first serial mating, a Tc+ form of the E. coli K12 Auxo strain was mated with the E. coli K12 Proto strain; in the second, a Tc+ isolate of the Proto strain from the first was mated with the original Tc- Auxo strain.

<table>
<thead>
<tr>
<th>Transfer rate* of Tc determinants in</th>
<th>No. of wild-type strains whose Tc determinants were transferred at these rates after they had been implanted in strain Auxo with</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st serial mating</td>
<td>F</td>
</tr>
<tr>
<td>$10^{-5}$ to $10^{-6}$</td>
<td>8</td>
</tr>
<tr>
<td>$10^{-6}$ to $10^{-5}$</td>
<td>6</td>
</tr>
<tr>
<td>$10^0$ to $10^{-1}$</td>
<td>8</td>
</tr>
</tbody>
</table>

* Expressed as the proportion of recipient organisms that acquired the determinant.

RESULTS

Mobilization of resistance determinants in wild-type strains of E. coli by different transfer factors

Most success in mobilizing resistance determinants was obtained with A2 and least with F (Table 1). Tc determinants in addition to Sm, Spc or Su determinants were mobilized in 14 wild-type strains but, with one exception, the subsequent implantation of the Tc determinants in strain Auxo occurred separately from that of the other determinants which were always implanted together. The 48 Auxo strains in which they (21) or Tc (27) had been implanted with I were fully susceptible to the If1 phage; all except three of the 42 Auxo strains in which they (1 of 20) or Tc (2 of 22) had been implanted with F were fully susceptible to the MS2 phage.
Transmission of F and I between E. coli K12 strains

In all the matings referred to below in which resistance determinants were transmitted between the E. coli K12 Auxo and Proto strains, F and I transferred at rates of 10^9 to 10^{-3}.

Transmission between E. coli K12 strains of Tc resistance determinants

In matings between the E. coli K12 Auxo strain carrying the 32 Tc determinants (implanted with F, I or A2) and the E. coli K12 Proto strain, and then between the resulting Tc+ Proto strains and the original Tc- Auxo strain, the Tc determinants were transmitted either at a very low rate in both matings, at a very low rate in the first mating and at a very high rate in the second mating, or at a very high rate in both matings (Table 2). Tc determinants which were transmitted at the high rate continued to be transmitted at that rate in subsequent matings. Ten were transmitted at the high rate in all matings mediated by F, I and A2; when five of them were re-mobilized from the wild-type strains with all three transfer factors and the first serial matings repeated, two were transmitted at the low rate by at least one of the transfer factors. In contrast, the Tc determinants of four wild-type strains, even after four serial matings, were still transmitted at a low rate by F, I and A2. All F+ recipient organisms tested from the high-rate Tc matings were Tc+ and all the F- ones were Tc-; a similar transfer factor/determinant relationship was also noted in all except two of the high-rate matings mediated by I.

To gain more information on the change from low-rate to high-rate Tc transmission, nine clones of an Auxo recipient strain carrying one of the Tc determinants, implanted with F, were mated with the Proto strain and one Tc+ Proto isolate from each of these nine matings was then mated with strain Auxo, and so on, until there had been six sets of matings between Auxo and Proto cultures. None of the nine Tc+ isolates from the first set of matings transmitted Tc at the high rate; the numbers from the second, third, fourth, fifth and sixth sets that did so were 6, 6, 7, 8 and 9 respectively.

In second serial matings in which the Tc determinants transferred at the low rate, but not in those in which they transferred at the high rate, some of the recipient Tc+ Auxo colonies were lactose-fermenting, indicating acquisition of the chromosomally-located lac genes of strain Proto. This was especially noticeable in transfers mediated by F; in many of these about 10 % of the Auxo colonies were lac+. Because of this finding, some of the first serial matings were repeated and Tc+ Proto isolates were checked to see whether they had also acquired the nal genes of the Auxo donor strains. This had occurred in many of the low-rate matings. In one mediated by F, 8 % of the Tc+ Proto isolates were nal+ and in another, 5 %. From one of these matings, 40 Tc+ Proto clones were mated with a spec instead of a nal mutant of strain Auxo. In all 40 matings, Tc transfer occurred at the low rate and in all of them 1 to 20 % of the Tc+ Auxo recipient organisms had acquired the lac genes of strain Proto. In another low-rate mating where the donor was a spec Proto strain in which the Tc determinants of one of the wild-type E. coli strains had been implanted with F, about 20 % of the Tc+ Auxo recipient organisms had acquired the spc determinants of strain Proto and about 7 % had acquired their lac determinants; about 20 % of the Auxo organisms in this mating that were selected for spectinomycin resistance were Tc+. Thus, in these crosses, the transfer of known chromosomal genes with Tc+ occurred more often than would be expected if they had been unlinked, i.e. if the Tc determinants were plasmid-borne. Mapping of Tc determinants in the chromosome was not undertaken, and when serial transfers of Tc+ were followed, clones that had received lac+ or nal+ with Tc+ were not used.
Antibiotic resistance determinants in E. coli

Table 3. Transfer rates of Sm, SmSu, SmSpc or SmSpcSu determinants of wild-type strains of E. coli between E. coli K12 strains in which they had been implanted with different transfer factors

In all matings which they mediated, F and I transferred at rates of $10^6$ to $10^{-1}$; the rate of A2 transfer was not assessed. The figures in parentheses indicate the numbers studied.

<table>
<thead>
<tr>
<th>Determinants</th>
<th>Transfer rate*</th>
<th>F</th>
<th>I</th>
<th>A2</th>
<th>F, I or A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm (4)</td>
<td>$10^6$ to $10^{-1}$</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>$10^{-5}$ to $10^{-4}$</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SmSu (14)</td>
<td>$10^6$ to $10^{-2}$</td>
<td>0</td>
<td>9</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>$10^{-3}$ to $10^{-4}$</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Nil</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SmSpc (8)</td>
<td>$10^6$ to $10^{-1}$</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>$10^{-5}$ to $10^{-6}$</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Nil</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>SmSpcSu (1)</td>
<td>$10^6$ to $10^{-1}$</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Expressed as the proportion of recipient organisms that acquired the determinant.

Sodium lauryl sulphate treatment eliminated F from six F+ Auxo strains that transmitted six different Tc determinants at the low rate and from six that transmitted the same determinants at the high rate. Tc was eliminated with F from the organisms of strains transmitting at the high rate but not from any of the organisms of the strains transmitting at the low rate.

Transmission between E. coli K12 strains of Sm, Spc and Su resistance determinants

In general, all three transfer factors transmitted Sm at high rates between the E. coli K12 Auxo and Proto strains but I and A2 transmitted SmSu at much higher rates than did F (Table 3). Most of the eight SmSpc determinants studied were transmitted at high rates by F and I; A2 transmitted four of them at high rates and four at low rates. The SmSpc determinants of two wild-type strains and the SmSu determinants of another one were not transmissible after they had been implanted with I in strain Auxo despite the fact that the three Auxo strains were fully susceptible to the T1 phage and I transferred from them at very high rates. The groups of determinants SmSu, SmSpc and SmSpcSu were always transmitted together.

Unlike Tc, the SmSu and SmSpc determinants that were transmitted at low rates in the first mating were transmitted at similar rates in all subsequent matings and there was no evidence of chromosomal genes being transmitted with them.

F+ and I+ antibiotic-sensitive recipient organisms were commonly isolated from all matings in which Sm and SmSu were transmitted by F and I. In contrast, in the high-rate SmSpc matings mediated by I, all the recipient organisms examined either possessed SmSpc and transfer factor or neither, suggesting that linkage had been established; similar results were obtained in the matings using three of the four high-rate transmitting F+ donors. Most of the organisms in these three SmSpc+F+ strains lost both SmSpc and F during sodium lauryl sulphate treatment but a few were isolated from each that were SmSpc+F-.
Transmission between E. coli K12 strains of a TcSmSpcSu determinant

The Tc, Sm, Spc and Su determinants of one wild-type strain, exceptional in that they had been mobilized en bloc by F, I and A2, were also transmitted en bloc in all matings between the Auxo and Proto strains. F+ donors transmitted these determinants at rates of approximately $10^{-2}$ or $10^{-4}$, irrespective of the particular rate at which they had acquired them in previous matings. I and A2 usually transmitted them at rates of $10^{-6}$ to $10^{-7}$. In some matings, however, rates of $10^0$ to $10^{-1}$ were recorded; organisms acquiring the determinants continued to transmit them at these high rates in subsequent matings. All the I+ recipient organisms tested from high-rate matings had acquired the determinants but none of the I- ones had; there was no such evidence of linkage between the determinants and F. Co-transfer of TcSmSpcSu and genes known to be chromosomally located (lac, nal and amp) was not detected in any of the F, I or A2 low-rate matings.

DISCUSSION

Nearly all the E. coli K12 Auxo strains in which the non-conjugative antibiotic resistance determinants of the wild-type E. coli strains had been implanted with F or I were fully susceptible to the MS2 or If1 phages. Thus, the possibility that the differing rates at which these determinants were subsequently transferred between the E. coli K12 strains was the expression of repressor genes co-transferred with the determinants of some of the wild-type strains during the mobilizing process can be discounted.

Some of the results of transmitting the Tc determinants by F, I and A2 resembled those obtained by Anderson & Lewis (1965) in mating experiments using a wild-type R factor-containing Salmonella typhimurium strain as donor. This strain, like many of our Tc+ Auxo strains, transmitted its Tc determinant to E. coli K12 at a very low rate and its transfer factor at a very high rate, but E. coli K12 exconjugants of this mating, like many of our E. coli K12 Proto Tc+ exconjugants isolated from matings with the low-rate transmitting Auxo strains, transmitted it at a very high rate due to linkage established between it and the transfer factor of the S. typhimurium strain.

In the present work, the available evidence indicated that the same Tc determinants occupied different attachment sites in the E. coli K12 donors from which they were transmitted at the low rate from those in donors from which they were transmitted at the high rate. In the donor transmitting at the high rate, certainly the F+ and I+ ones, these determinants occupied the extrachromosomal site of the transfer factors with which they had become linked. However, in the donor transmitting at the low rate (and, presumably, in the wild-type strains from which they were derived) the evidence suggests that Tc resistance determinants were chromosomally located, because genes known to be chromosomally located were often transmitted with them; during subsequent matings, recombination with the transfer factors occurred and Tc became plasmid-borne. Harada et al. (1964) have demonstrated this with E. coli K12 organisms in which Tc determinants had been chromosomally integrated by transduction and then infected with F. Such a recombination event need only be a rare occurrence because organisms possessing plasmid-determined tetracycline resistance would be selected in our mating experiments due to the much higher transfer rate of plasmid-borne than chromosomal-borne Tc. Even so, the Tc determinants of four of the wild-type strains were never transmitted at a high rate by F, I or A2. In contrast, the Tc determinants of a further 10 of the wild-type strains were always transmitted at high rates by all the transfer factors with which they had been mobilized. These
particular Tc determinants could have been located chromosomally or extrachromosomally in the wild-type strains, in the former case re-combining with the transfer factor and assuming an extrachromosomal location during the mobilization process. Supporting evidence for a chromosomal location was obtained from the observation that when the determinants in five of the ten strains were re-mobilized with the three transfer factors, the determinants of two of them were subsequently transmitted by at least one transfer factor at the low rate.

The absence of linkage between transfer factor and Sm and SmSu determinants in the E. coli K12 strains enabled differences to be detected between transfer factors and between determinants. For example, F transmitted SmSu at much lower rates than I or A2 while Sm was transferred by F at much higher rates than SmSu. SmSpc, SmSpcSu and TcSmSpcSu determinants, however, resembled the Tc determinants in that many of them became linked to the transfer factors in the E. coli K12 strains and, consequently, were transmitted at the usual high rates of the transfer factors. The linkage between F and SmSpc was certainly not as firm as that established between F and Tc in that some SmSpc-F- organisms were occasionally found amongst the SmSpc-F- ones obtained by treating E. coli K12 SmSpc-F+ strains with sodium lauryl sulphate; F- organisms isolated from F and Tc-linked E. coli K12 strains similarly treated were invariably Tc-. The low rate of SmSpc transfer from four E. coli K12 A2+ strains and one F+ strain and its association with a probable lack of determinant-transfer factor linkage (in the case of the F+ strain) was another example of the resemblance between SmSpc and Tc determinants. However, the similarity appeared to end here because the SmSpc determinants in these five strains continued to be transmitted at low rates in all subsequent matings between the Proto and Auxo strains and always without any evidence of the co-transfer of genes known to be chromosomally located.

Resistance to spectinomycin and streptomycin in the strains designated SmSpc+ was probably due to synthesis of the adenylylating enzyme that inactivates both antibiotics as distinct from the phosphorylating enzyme that inactivates only spectinomycin (Ozanne et al., 1969; Benveniste, Yamada & Davies, 1970) and which, presumably, was responsible for the streptomycin resistance of the strains designated Sm+ and SmSu+. These latter strains were more resistant to streptomycin than the SmSpc+ strains; a similar difference was found by Pinney & Smith (1974) between a known phosphorylating strain and two known adenylylating strains of E. coli K12.

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REFERENCES


