Genetic Heterology between *Escherichia coli* k12 and a Smooth Strain of *E. coli*

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**SUMMARY**

CF 2004-6, a recipient strain, and 04 M 2, an Hfr donor strain of *Escherichia coli* 04:K(undetermined):H5, were used in mating experiments with *E. coli* K12. 04 M 2 behaved like the K 12 donor strain in mating experiments. These donor strains genetically recombined with the K12 recipient at higher frequencies than with CF 2004-6. The results of interrupted mating experiments were also more typical with the K 12 recipient than with CF 2004-6 in that there were sharp entry times for the markers studied and linear increases of recombinants with time. The possibility that host-controlled restriction decreased genetic recombination between *E. coli* K12 and CF 2004-6 was pursued. We obtained no evidence for this possibility. Instead the data suggest that genetic heterology between these strains is affecting recombination. Genetic heterology also explains why 04 M 2 behaves more like a K 12 donor in mating experiments with CF 2004-6.

**INTRODUCTION**

Smooth strains of *Escherichia coli*, i.e. those which are known to contain O and K antigens, can be sexually competent (Ørskov & Ørskov, 1961, 1966; Ketyi & Szendrei, 1967). Many if not the majority of the freshly isolated strains used in early fertility surveys (Lederberg, Cavalli & Lederberg, 1952; Furness & Rowley, 1957) and more recently for transfer of a variety of plasmids (Meynell, Meynell & Datta, 1968) were probably smooth. However, most of these strains were used only to establish donor or recipient ability or to study the transfer of a limited number of markers attached to the plasmids. When CF2004-6, a strain of *E. coli* 04:K(undetermined):H5, was shown to be a recipient (Zubrzycki & Spaulding, 1965) it became of interest to see how this typical smooth strain behaved in mating experiments. We found that it did not mate in broth, possibly because surface antigens prevented effective contact formation. The Millipore filter technique described in the Methods section allowed us to use CF2004-6 in kinetic mating experiments. In preliminary work we became convinced that the genetic map of our smooth strain of *E. coli* would be similar to that established with K12 (Taylor & Trotter, 1967). We did find that the recombination frequencies in *E. coli* K12 × CF2004-6 matings were lower than those in K12 × K12 matings. Because host-controlled restriction is the cause of low recombination frequencies between *E. coli* K12, and *E. coli* B strains (Boyer, 1964; Copeland & Bryson, 1966) and *E. coli* K12 (P1) (Arber & Morse, 1965; Colson, Glover, Symonds & Stacey, 1965), we decided to check whether it occurred in our studies. The data presented in this paper suggest that genetic heterology (some type of dissimilarity between DNA which
hinders recombination) rather than restriction accounts for the low recombination frequencies between \textit{E. coli} \textit{K}12 and CF2004–6 strains.

\section*{Methods}

\textbf{Media.} Penassay broth (antibiotic medium No. 3, Difco) was used for the cultivation of the organisms. The minimal agar was essentially the Davis minimal medium prepared as described by Lederberg (1950) except that it contained 0.5\% glucose and approximately 1.3\% Noble agar (Difco). The amino acids were used at a concentration of 30 to 40 \(\mu\text{g.}/\text{ml.}\) Streptomycin was used at a concentration of 500 \(\mu\text{g.}/\text{ml.}\) to counterselect against the donor strains. Minimal enriched agar was minimal agar enriched with 0.2\% Penassay broth.

\textbf{Bacteria.} Our recipient smooth strain of \textit{Escherichia coli} is labelled CF2004–6. Mutants of CF2004–6 were isolated after treating a 24 hr culture with 0.1 M-ethyl methane sulfonate for 1 hr. An Hfr donor strain, 04M2, of CF2004–6 was obtained by selection for a terminal marker, lactose, in a mating between \(\chi 4x6\) and Lac 6. All these strains plus the \textit{K}12 strains are listed in Table 1.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
Strain designation & Strain origin & Characteristics* & Sex \\
\hline
LZ–TM–6 & CF 2004–6 & \textit{trp, leu, arg, his, pro, str-r} & F– \\
LZ–TM–9 & CF 2004–6 & \textit{trp, leu, arg, his, thr, pro, str-r} & F– \\
Lac 6 & CF 2004–6 & \textit{trp, ilv, lac} & F– \\
04M2 & CF 2004–6 & \textit{trp, ilv} & Hfr \\
P4X6 & K12 & \textit{met} & Hfr \\
HfrH & K12 & \textit{wild type} & Hfr \\
AB1133 & K12 & \textit{thr, leu, arg, his, pro, thi mal, gal, lac, mil, xyl, str-r} & F– \\
\hline
\end{tabular}
\caption{Strains of \textit{Escherichia coli} used}
\end{table}

* In the text, reference will also be made to the host-controlled restriction and modification locus which is designated as \textit{hsp} (Taylor & Trotter, 1967).

The order of transfer of markers from \(\chi 4x6\) and 04M2 in a counterclockwise direction is \textit{0-pro-leu-thr-arg-his . . . lac}. The order of transfer of markers from HfrH in a clockwise direction is \textit{0-hsp-thr-leu-pro-his . . . arg}.

\textbf{Millipore filter matings.} CF2004–6 strains do not conjugate well in broth. We therefore modified the Millipore mating technique described by Matney & Achenbach (1962) and Sanderson (1967). Overnight cultures of donor and recipient were diluted 1/100 in Penassay broth. The female culture was incubated in a 37° water bath shaker for 3 hr and reached 5 to 8 \(\times 10^6\) viable bacteria per ml. The donor culture was incubated for 3 hr in a 37° water bath without shaking and reached 3 to 5 \(\times 10^6\)/ml. Nine ml. of the female culture and 1 ml. of the male culture were mixed. The female–male mixture was kept in an ice bath to prevent growth and mating. One ml. samples were filtered on to 27 mm. Millipore filters (type HA). The filter was transferred to 2 ml. of minimal glucose broth in a tube standing in a 37° water bath. Timing started at this point. All matings were terminated by removing the tubes from the water bath, adding 8 ml. of ice-cold saline and agitating the tube on a Vortex Jun. mixer (maximum speed) for approximately 80 sec. Appropriate dilutions were made in saline and 0.1 ml. samples were placed into Petri dishes. Melted minimal agar kept at approximately 50° was poured into each dish and mixed.
Genetic heterology between *E. coli* strains thoroughly with the 0.1 ml. samples. After hardening, the agar plates were incubated at 37° for 48 hr. This pour plating technique gave us higher recombinant frequencies and more reproducible results than spreading samples on the surface of agar.

**Linkage analyses.** Linkage between markers was calculated using the following formula:

\[
\frac{\text{no. recombinants for markers } a \text{ and } b}{\text{no. recombinants for marker } a} \times 100 = \% \text{ a}^+ \text{ recombinants that are also } b^+.
\]

One tenth ml. samples of the recombinant mixture were plated in agar lacking one amino acid in order to determine the number of recombinants which received one marker and in agar lacking two amino acids for double recombinants. Susan U. Levinson (unpublished) found this technique gave results comparable to those obtained by selecting approximately 200 colonies and scoring for unselected markers on appropriate minimal agar. The frequencies obtained by this technique should be much more reliable because of the greater numbers involved in the analyses.

**Marker stability of *Escherichia coli* recombinants.** Unstable heterogenotes occur among hybrids of enteric bacilli (Falkow, Rownd & Baron, 1962; Luria & Burrous, 1957) and even among recombinants of *Escherichia coli* (Lederberg, 1949; Ketyi & Szendrei, 1967). All recombinants to be used in backcrosses were tested for marker stability by streaking on minimal agar, incubating for 48 hr and then restreaking on minimal enriched agar. Reconstruction experiments using known unstable *E. coli* K12-CF2004-6 recombinants (none of which occurred in this study) showed that they grew out in 48 to 72 hr as a mixture of regular sized and tiny colonies.

### RESULTS

In preliminary mating experiments between *Escherichia coli* K12 and CF2004-6 we found lac, pro, leu, thr, arg, ilv, mal, his and trp markers to be located in CF2004-6 where expected, according to the genetic map established with *E. coli* K12 (Taylor & Trotter, 1967). In addition to the linkages reported in detail here, we found xyl closely linked to the str locus and ilv to a rough locus, possibly the *E. coli* equivalent of the rou A locus described in *Salmonella typhimurium* (Subbaiah & Stocker, 1964). These results convinced us that the genetic map of our smooth strain of *E. coli* was similar to that of *E. coli* K12. However, genetic recombination in these inter-strain matings occurred at low frequencies. We decided to test whether host-controlled restriction could account for these low frequencies as it undoubtedly does in matings between rough strains of *E. coli*.

In matings involving the K12 donor P4X6 and the CF2004-6 recipient Lz-TM-6, one obtains lower recombinant frequencies and decreased linkage between markers when compared with an intra-strain K12 × K12 mating using AB1133 as a recipient, Table 2 (crosses 1 and 2) and Table 3 (Test 1). Since 04M2 is a derivative of CF2004-6, we now expected higher recombinant frequencies and increased linkage between markers when it conjugates with Lz-TM-6. The results (Table 2, crosses 3 and 4) show that except for the arginine marker 04M2 recombines with Lz-TM-6 and AB1133 at frequencies comparable to those using P4X6 (crosses 1 and 2). Explanations for these results will be offered later, but at this time it should be pointed out that the higher recombinant frequency for arg indicates that the lower recombinant frequencies for other markers observed with Lz-TM-6 were not due to it being a poor recipient. The
results of linkage analyses similarly show that 04M2 behaved like P4X6 (Table 3, Tests 1 and 2). The exception again was with the arg marker of LZ-TM-6 which showed increased linkage to pro (from 3.9 to 10%) and leu (from 2.4 to 25%) in matings with 04M2. The results of interrupted mating experiments (Fig. 1 to 4) are expressed using different scales because of the range in recombinant frequencies, but this cannot

Table 2. Recombinant frequencies after 100 min. matings

<table>
<thead>
<tr>
<th>Cross</th>
<th>Donor × Recipient</th>
<th>Pro±</th>
<th>Leu±</th>
<th>Arg±</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P4X6 × AB1133</td>
<td>23.0</td>
<td>27.0</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>P4X6 × LZ-TM-6</td>
<td>0.22</td>
<td>2.6</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>04M2 × AB1133</td>
<td>19.0</td>
<td>18.0</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>04M2 × LZ-TM-6</td>
<td>0.02</td>
<td>2.8</td>
<td>0.26</td>
</tr>
<tr>
<td>5</td>
<td>P4X6 × H3</td>
<td>0.23</td>
<td>4.3</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>P4X6 × H1</td>
<td>0.27</td>
<td>3.8</td>
<td>0.27</td>
</tr>
<tr>
<td>7</td>
<td>HfrH × AB1133</td>
<td>28.0</td>
<td>48.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>HfrH × LZ-TM-6</td>
<td>1.3</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>HfrH × H3</td>
<td>6.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>HfrH × H1</td>
<td>6.6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>P4X6 × H4</td>
<td>8.8</td>
<td>2.9</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The average recombinant frequencies from repeat mating experiments is shown in crosses 1, 2, 3, 4, 7 and 8. Only the average recombinant frequencies from backcrosses with individual K12-CF2004-6 hybrids is shown in crosses 5, 6, 9, 10 and 11 because the individual frequencies varied no more than those observed in repeat mating experiments using the Millipore technique as described in the Methods section.

Table 3. Linkage from 100 min. inter- and intra-strain matings

<table>
<thead>
<tr>
<th>Donor</th>
<th>Test</th>
<th>Pro</th>
<th>Leu</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AB1133</td>
<td>LZ-TM-6</td>
<td>AB1133</td>
</tr>
<tr>
<td>P4X6</td>
<td>1</td>
<td>100.0</td>
<td>100.0</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>58.0</td>
<td>4.3</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>2.8</td>
<td>37.0</td>
<td>52.0</td>
</tr>
<tr>
<td>04M2</td>
<td>2</td>
<td>100.0</td>
<td>100.0</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>62.0</td>
<td>6.3</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>33.0</td>
<td>1.8</td>
<td>34.0</td>
<td>16.0</td>
</tr>
<tr>
<td>HfrH</td>
<td>3</td>
<td>100.0</td>
<td>100.0</td>
<td>83.0</td>
</tr>
<tr>
<td></td>
<td>67.0</td>
<td>47.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Only the average per cent linkages from backcrosses with different hybrids is shown because the individual results did not vary much from the average. The per cent linkage between two markers was determined by comparing the number of recombinants which received the two markers to those which received one of the markers which is expressed in the table as 100%. This was done after a 100 min. mating by plating aliquots of the recombinant mixture in agar lacking two amino acids in order to determine the number of double recombinants and in agar lacking one amino acid for single recombinants.

affect the interpretation of the data which show the appearance of markers at about the same time in each cross. Note the non-linear appearance of markers in Fig. 3 and 4. The non-linear appearance of markers during conjugation was shown to be associated with host-controlled restriction by Copeland & Bryson (1966). However, the same type of curve has even been observed between Escherichia coli K12 strains, P4X6 and
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AB1133 (Schneider & Falkow, 1964). Therefore, a non-linear curve is no indication that restriction is occurring. The great delay in the appearance of markers observed by Boyer (1964) is an obvious manifestation of restriction, but this did not occur in our crosses. Nevertheless, host-controlled restriction could still account for the lower recombinant frequencies and reduced linkages obtained with LZ-TM-6. If this is so,

Fig. 1. Interrupted mating experiment between P4X6 and AB1133. One ml. mixtures of log. phase donor (3 to 5 x 10^9 ml.) and recipient (5 to 8 x 10^9/ml.) cultures were filtered on to Millipore filters. The filters were placed in tubes containing minimal glucose broth which were incubated at 37°. At time intervals cold saline was added to the tubes which were then shaken on a vortex Jun. mixer in order to interrupt the matings. Samples were now plated in order to select for Pro^+ (△—△), Leu^+ (■—■) and Arg^+ (○—○) recombinants. For details see Fig. 1.

Fig. 2. Interrupted mating experiment between O4M2 and AB1113. Pro^+ (△—△), Leu^+ (■—■) and Arg^+ (○—○) recombinants. For details see Fig. 1.

Fig. 3. Interrupted mating experiment between P4X6 and LZ-TM-6. Pro^+ (△—△), Leu^+ (■—■) and Arg^+ (○—○) recombinants. For details see Fig. 1.

Fig. 4. Interrupted mating experiment between O4M2 and LZ-TM-6. Pro^+ (△—△), Leu^+ (■—■) and Arg^+ (○—○) recombinants. For details see Fig. 1.

then incorporation in a CF2004-6 recipient of the K12 host-controlled restriction and modification locus (K12-hsp locus) should make them appear more K12-like in matings. This work was pursued using only K12 donor strains since we knew that restriction and modification occur with K12 strains. The approach we took was similar to that
used by Boyer (1964), who found linkage between the \textit{hsp} locus and the \textit{thr} marker. An HfrH × LZ-TM-9 mating was interrupted at 6, 9 and 12 min. From the various time intervals a total of eighteen Thr$^+$ recombinants (labelled H3) was isolated. The recombinants were checked for stability and then backcrossed with P4X6 (Table 2, cross 5). There was no marked increase in recombinant frequencies for \textit{pro} or \textit{arg} when compared to the results shown in cross 2, but there did seem to be a slight increase for \textit{leu}. Linkage analyses were done on all crosses (Table 4, Test 1 with H3). Compared to the results using LZ-TM-6 (Table 3, Test 1 with LZ-TM-6), there was an increase in linkage for \textit{leu} both among Pro$^+$ (from 31 to 58\%) and Arg$^+$ (from 12 to 26\%) recombinants. That these slight increases for \textit{leu} recombination and linkage may not be specifically related to the K12-hsp locus is suggested by the following experiments.

### Table 4. Linkage from 100 min. backcrosses with recipient \textit{K12-CF2004-6} hybrids

<table>
<thead>
<tr>
<th>Donor</th>
<th>Test</th>
<th>Recipient</th>
<th>Pro</th>
<th>Leu</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4X6</td>
<td>1</td>
<td>H3</td>
<td>100%</td>
<td>58%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>H3</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>HfrH</td>
<td></td>
<td>H1</td>
<td>100%</td>
<td>26%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4</td>
<td>23%</td>
<td>20%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H3</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H1</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Only the average per cent linkages from backcrosses with different hybrids is shown because the individual results did not vary much from the average. The per cent linkage between two markers was determined by comparing the number of recombinants which received the two markers to those which received one of the markers which is expressed in the table as 100\%. This was done after a 100 min. mating by plating samples of the recombinant mixture in agar lacking two amino acids in order to determine the number of double recombinants and in agar lacking one amino acid for single recombinants.

A P4X6 × LZ-TM-6 conjugation was interrupted at 6 and 9 min. in order to get Pro$^+$ recombinants. Eleven Pro$^+$ recombinants (labelled H1) were isolated, checked for stability, found to be \textit{leu} negative and presumed to be K12-hsp negative since this locus is even distal to \textit{leu}. The recombinant frequencies from backcrosses with P4X6 and these recombinants are shown in Table 2. Compared to the results using LZ-TM-6 (cross 2), backcrosses using H1 (cross 6) resulted in a slight increase for Leu$^+$ recombinants similar to that using H3 (cross 5). Since H1 is Pro$^+$, the only linkage determined was that between \textit{arg} and \textit{leu} which is shown in Table 4 (Test 1 with H1). Compared to the results using LZ-TM-6 (Table 3, Test 1 with LZ-TM-6), there was a slight increase in this linkage (from 12 to 23\%) similar to that observed with the H3 recipients (from 12 to 26\%). Another \textit{E. coli} K12 donor strain, HfrH, was used in experiments similar to those just described for P4X6. The recombinant frequencies in inter-strain matings were considerably lower than those in intra-strain matings as shown in Table 2 (crosses 7 and 8). In spite of this, linkage between \textit{pro} and \textit{leu} in the inter-strain matings was not greatly affected (Table 3, Test 3, compare the results using AB1133 and LZ-TM-6). Backcrosses with six H3 recombinants resulted in some increase in recombinant frequencies for \textit{pro} and \textit{leu} (Table 2, compare crosses 8 and 9). Linkage analyses on these backcrosses are shown in Table 4 (Test 2 with H3). Compared to the
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results using LZ–TM–6 (Table 3, Test 3 with LZ–TM–6), selection for the distal marker pro resulted in an increase in linkage with leu (from 53 to 68%). However, when selecting for the proximal marker leu there was no increase in linkage. HfrH was also backcrossed with three H1 recombinants. Compared to the results using LZ–TM–6 (Table 2, cross 8), backcrosses using H1 (cross 10) resulted in a slight increase for Leu+ recombinants similar to that using H3 (cross 9). Since H1 is Pro+, the recombinant frequency and linkage for this marker could not be determined.

In all these backcrosses using K12–CF2004–6 recombinants slight increases in recombinant frequencies and linkage could be associated with the incorporation of the K12–thr marker which is closely linked to the K12–hsp locus. But that does not mean that the K12–hsp locus is functioning in CF2004–6 because similar increases were also found with recombinants which contained a K12 region (pro) not closely linked to the K12–hsp locus. Our interpretation for these slight increases is that the presence of a piece of K12 DNA in CF2004–6 increases the chances of crossing over between this K12 DNA and the homologous DNA region in the E. coli K12 donor, thus increasing genetic recombination. Take, for example, the results obtained from matings between E. coli K12 and H3. The slight increases for Leu+ recombinant frequency and linkage are probably due to the presence of the adjacent K12 thr region in H3. To support this interpretation we present the results of backcrosses with two typical H1 recombinants which were mutated at the K12 pro region (labelled H4). Backcrosses involving P4X6 and H4 show a higher Pro+ recombinant frequency when compared with a mating using LZ–TM–6 (Table 2, crosses 2 and 11). Linkage analyses on these crosses are shown in Table 4 (Test 1 with H4). Compared to the results using LZ–TM–6 (Table 3, Test 1 with LZ–TM–6), there was an increase in linkage for pro (from 4.3 to 43%) when selecting for leu and increases for pro (from 2.8 to 19%) and leu (from 12 to 20%) when selecting for arg. These results show that areas of DNA homology do increase the chances of recombination between strains of E. coli.

**DISCUSSION**

The data presented in this paper suggest that restriction plays no obvious role in genetic recombination between Escherichia coli K12 and CF2004–6 strains for the following reasons. In spite of lower recombinant frequencies and decreased linkage between genes, there was no delay in appearance of markers. Backcrosses with eighteen E. coli K12–CF2004–6 recombinants which received the thr locus and presumably the K12–hsp locus from HfrH showed no more of an increase in recombinant frequencies and linkage than did backcrosses with recombinants which received from P4X6 the pro locus, which is not linked to hsp.

Boyer (1964) found that the K12–hsp locus was incorporated along with the thr marker in 60% of the Escherichia coli b/r recombinants. It may be argued that this and linkage is greatly reduced in CF2004–6 strains. As shown in Table 3, linkage of leu pro in HfrH × CF2004–6 matings was decreased not even twofold when compared with linkage in HfrH × ABI33 matings. This difference is even less than that reported for the same two markers in E. coli HfrH × b/r matings (Boyer, 1964). Therefore, it seems likely that the closely linked markers, hsp and thr, would both be incorporated in CF2004–6 in as high a percentage of cases as they were in E. coli b/r.

We attribute the lower recombination frequencies to genetic heterology between
Escherichia coli K12 and CF2004–6 and the increase in these frequencies upon back-crossing with these inter-strain recombinants to the artificially established genetic homology. This interpretation is the same as that invoked by Johnson, Falkow & Baron (1964) to explain the lower recombination frequencies between E. coli and Salmonella typhi and the increase in frequencies upon rematings with hybrids. Genetic heterology can also be used to explain why E. coli 04M2, an Hfr derivative of CF2004–6, gives higher recombination frequencies with the K12 recipient than with CF2004–6. In deriving this strain (see Methods), not only did the E. coli K12 lac region and the F factor become incorporated into the CF2004–6 genome but possibly the pro to leu region as well. The arg region did not, and that is why there is more genetic recombination for this region between 04M2 and CF2004–6. This hypothesis is supported by experiments (unpublished) in which we use transductions by Φ04–CF to distinguish between the genes of E. coli K12 and CF2004–6 (Zubrzycki & Gainsburg, 1966).

During the process of the work reported here, we realized that the restriction mechanism is probably a surface phenomenon (Schell & Glover, 1966). In this regard, it was suggested that the Millipore mating technique, by artificially forcing bacteria together, bypassed the normal surface opposition of donor and recipient and consequently the restriction mechanism. We obtained E. coli B/R strains from Boyer and demonstrated that our mating technique would allow the restriction mechanism to be expressed. We also proved with his strains that our laboratory strain of HfrH contained and could successfully transmit the K12-hsp locus to E. coli B/R. We also entertained the possibility that the surface antigens on our smooth strain may be interfering with a surface phenomenon such as restriction. We isolated four rough strains of LZ–TM–6. In preliminary experiments we did not observe restriction during conjugation. These experiments were discontinued when a complete host-controlled restriction and modification system was discovered in our smooth strains (to be published). This system is accompanied by radical effects on genetic recombination and, restriction and modification of phage Φ04–CF (Zubrzycki, Green & Spaulding, 1966). Preliminary experiments with this E. coli 04 restriction and modification system prove that the K–12 hsp locus is closely linked to thr in matings with CF2004–6. However, in the experiments reported here the K12–hsp locus could not function in CF2004–6. It has been speculated that the restriction and modification phenomenon is of benefit to a strain of bacterium because it allows genetic exchange with its own strain but prevents it with another (Wood, 1966). The results presented in our studies suggest that the occurrence of restriction and modification in genetic experiments between bacterial strains is unpredictable and the true evolutionary value of the phenomenon is obscure.

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