DRUG RESISTANCE OF ENTERIC BACTERIA

III. ACQUISITION OF TRANSFERABILITY OF NONTRANSMISSIBLE R(TC) FACTOR IN COOPERATION WITH F FACTOR AND FORMATION OF FR(TC)

KENJI HARADA, MITSUO KAMEDA, MITSUE SUZUKI, AND SUSUMU MITSUHASHI

Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan

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ABSTRACT

Harada, Kenji (Gunma University, Maebashi, Japan), Mitsu Kameda, Mitsue Suzuki, and Susumu Mitsuhashi. Drug resistance of enteric bacteria. III. Acquisition of transferability of nontransmissible R(TC) factor in cooperation with F factor and formation of FR(TC). J. Bacteriol. 88:1257-1265. 1964.—Transmissible drug-resistance factor R, which confers resistance to tetracycline, chloramphenicol, streptomycin, and sulfonamide, was previously found to be transduced in the system of Salmonella E group with phage epsilon. The R factor of R+ transductants was nontransmissible by cell-to-cell contact, and it was not eliminated by treatment with acridine dye. When R+ transductants were infected with F factor, the nontransmissible R factor acquired transferability by conjugation. The R+ conjugants, to which only the R factor was separately transmitted by conjugation from the (F+R+) donor, were still unable to transfer their R factor by conjugation. However, the (FR)+ conjugants, to which both F and R factor were transmitted simultaneously by conjugation, were also capable of transferring their F and R factors by conjugation. From the present study, it was concluded that the recombinant (FR) factor was formed as a result of an interaction between F and R factors present in a host bacterium, and that one of the mechanisms of acquisition of transferability is accounted for by the formation of recombinant (FR) factor. The recombinant (FR) factor was transferable by conjugation, and it conferred both the drug-resistance and F+ characters to the recipient cells. The (FR) factor was eliminated by treatment with acridine dye and also transduced as one unit into Escherichia coli K-12 by P1Ke phage.

The transmissible drug-resistance R factors were able to transfer their drug resistance by cell-to-cell contact. When two types of R factor were brought together in a host bacterium by superinfection, recombinant R factors were formed as a result of interaction between these two types (Mitsuhashi et al., 1962). We previously reported that R factors could be transduced in the system of Salmonella E group with phage epsilon (Harada et al., 1963). Also, we found that the R factor of R+ transductants is nontransmissible by conjugation and cannot be eliminated by treatment with acriflavine, whereas the R factor that was transferred by conjugation was eliminated by acriflavine treatment (Mitsuhashi, Harada, and Kameda, 1960, 1961a, b).

The previous paper reported that the nontransferable R factor of R+ transductant, obtained with epsilon phage, acquired transmissibility after infection with F factor (Harada et al., 1963). This paper deals with the mechanism involved in the acquisition of transferability of nontransmissible R factor in cooperation with F factor.

MATERIALS AND METHODS

Microorganisms. Salmonella newington (S-84) was used in this study. Its somatic antigens and lysogenicity with epsilon phages were described previously (Harada et al., 1963). The following strains of Escherichia coli K-12 were used: E. coli K-12 strain W1177 F⁻ which is auxotrophic for threonine (T), leucine (L), and thiamine (B₁), and possesses the markers lac⁻, ml⁻, mal⁻, ara⁻, xyl⁻, gal⁻, and SM⁻; E. coli K-12 strain W4574 F⁻ which is auxotrophic for proline (pro) and has the markers lac⁻, ml⁻, mal⁻, ara⁻, xyl⁻, gal⁻, and SM⁻; E. coli K-12 strain 58-161 F⁺ and F⁺ which are auxotrophic for methionine (M); E. coli K-12 strain W4861 F⁺ which is auxotrophic for purine (pur) and possesses the markers lac⁻ and T₄⁺; and E. coli K-12 strain W3747 F₁₁⁺ which is auxotrophic for methionine (M).

The F⁺ bacteria can transfer certain extra-chromosomal (F-linked) genes, with a high
frequency, to F⁻ (Jacob and Adelberg, 1959; Hirota, 1959). The F₁₈ factor was isolated by Hirota (1959). F₁₈⁺ denotes a F⁺ strain, and it is able to transfer with high frequency lac⁺, pur⁺, T₄⁺, and P⁺ (ability to synthesize alkaline phosphatase). In this paper, F⁺ (lac) denotes the F₁₈⁺ strain, and the lac⁺ marker is the only marker pertinent to the experiments. E. coli K-12 strain W1367 Hfr is a F⁺ variant capable of transferring the bacterial chromosome with high frequency, and it has the markers M⁻, lac⁻, SM⁺. Shigella flexneri 2b was used as a recipient of R factor.

**Phages.** E. coli K-12 and phage P1kc (Lennox, 1955) were used in the transductional system. The phages f₁ and f₂ (Loeb, 1960; Loeb and Zinder, 1961) were used to check for the presence of F factor in F⁺ or F⁻ strains. Epsilon 15 (Iseki and Sakai, 1953) and epsilon 34 (H harada, 1956a, b) phages were used as the transducing phages. These two phages are able to confer the ability to form O-15 and O-34 antigens to the host bacteria when lysogenized. ε₁₄₁ phage is a mutant derived from ε₁₄, and it is unable to convert antigenically the host bacteria from O-15 to O-34 when lysogenized (Harada et al., Japan. J. Microbiol., in press).

**R factors.** R₃₂(TC) and R₄₀(TC) factors were obtained by separation of the R₁₀(TC.CM.SM.SA) factor upon transduction with ε₁₄ and ε₁₄₁ phages, respectively (Harada et al., 1963). R₁₁(TC) factor was obtained by separation of R₁₀(TC.CM.SM.SA) factor during transduction with ε₂₄₁ phage. The R₁₁(TC), R₂₃(TC), and R₄₀(TC) factors were all nontransmissible by conjugation, and they were not eliminated by treatment with acriflavine.

**Media.** Liquid cultures were propagated in Brain Heart Infusion (BHI, Difco) broth. The nutrient agar was prepared from Heart Infusion (HI) Agar (Difco) and bromothymol blue-lactose agar (containing 1% lactose). L broth-agar was prepared as described by Lennox (1955), and was used for the propagation of f₁ and f₂ phages. The minimal agar consisted of medium A (Davis and Mingioli, 1950) without sodium citrate but containing 0.04% eosin and 0.065% methylene blue (EM agar). Amino acid supplements, when necessary, were added to a concentration of 20 mg per liter. Thiamine hydrochloride and adenine (ade) were added to the EM agar, when necessary, to a concentration of 10 and 20 mg per liter, respectively. Sugars were supplied to the base medium at a concentration of 10 g per liter. Mueller-Hinton agar (Difco) was used for a nutrient medium in selecting sulfonamide (SA)-resistant clones and in determining SA resistance. The EMG Ga agar medium consisted of EM agar containing 1.0% glucose and 0.5% sodium glutamate. The EM Lac Ga agar medium consisted of EM agar containing 0.5% sodium glutamate and 1.0% lactose instead of glucose. The EM Lac Asp agar medium consisted of EM agar containing 1.0% lactose and 0.1% l-asparagine instead of sodium glutamate.

Drugs, determination of drug resistance, transmission of R factors by mixed cultivation, and conditions of transduction were described in the preceding paper (Harada et al., 1963).

**Acriflavine orange treatment.** The elimination of R factors by treatment with acridine orange was carried out according to the method described by Hirota (1960), and was reported previously (Mitsuhashi et al., 1961a, b). BHI broth containing 5, 10, 30, 40, and 60 μg/ml of acridine orange was used for the elimination of R and F factors. The medium was adjusted to pH 7.6 by adding 1 N NaOH. The microorganisms (ca. 10⁶ per ml) to be tested were inoculated in each tube containing the acridine dye and incubated for 24 hr at 37 C. The tube which showed maximal growth was diluted with saline, and 0.1 ml of an appropriate dilution was plated on bromothymol blue-lactose-agar. After incubation at 37 C for 18 hr, the presence of F and R factors in cells of each colony that developed on the plate was examined. As a control, BHI broth without acridine dye was used to check for the spontaneous loss of F and R factors.

**Test of F⁺ and R⁺ microorganisms.** R⁺ microorganisms were detected by their growth on HI Agar containing tetracycline (TC, 25 μg/ml), chloramphenicol (CM, 25 μg/ml), or streptomycin (SM, 25 μg/ml), and by transmissibility of drug resistance by conjugation. In the case of nontransmissible drug-resistance factor, the transmissibility was tested by infection with F factor. F⁺ microorganisms were detected by sensitivity to f₁ and f₂ phages, and by their ability to mate with a F⁻ strain. The F₁₈ factor was detected by lac⁺ character in accordance with Fduction.

Transduction with P1 phage was conducted according to the method described by Lennox (1955).

**Blender treatment.** A sample was withdrawn.
from the mixed culture of donor and recipient strains every 5 min and diluted 1:1,000 with medium A, containing 5.0% sodium glutamate and 1.0% lactose. The diluted sample was treated in a blender at 5,000 rev/min for 15 sec in the cold and allowed to stand for 30 min at 37 C. A 0.1-ml amount of this material was then added to 3.0 ml of medium A containing 20 \( \mu g/ml \) of adenine and 0.5% agar, which was kept in the melted state at 45 C. The contents of the tube were poured onto an appropriate selective medium. After solidification of the second layer of agar, the plate was incubated for 48 hr at 37 C, and the transmission frequencies of F(lac) and R(TC), the sexuality and the drug resistance of the recipient strain, were determined.

**RESULTS**

*Transmission of F(lac) factor to R* \(^+\) transductants of *S. newington* (S-84). In the previous paper (Harada et al., 1963a), it was reported that the R factor of R* transductants in *S. newington* and *S. chittagong* was nontransmissible by conjugation, and that the R factor was not eliminated by treatment with acriflavine. To determine the reason for the nontransmissibility of the R factor of R* transductants in the *Salmonella* E group, F(lac) factor was transferred and a study was made on the acquisition of transmissibility of otherwise nontransferable R factor. The F(lac) factor of *E. coli* W3747 F+(lac) was transferred by mixed cultivation to R* transductants of *S. newington*. As shown in Table 1, F(lac) factor was transmitted to *S. newington* (S-84)E, R44* and (S-84)E, R41* at a transmission frequency of \( 10^{-7} \)

<table>
<thead>
<tr>
<th>Recipient in cross</th>
<th>Transmission of F(lac) factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S-84)E1, R51*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E2, R31*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E3, R4*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E4, R6*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E5, R6*</td>
<td>+(-7.0)</td>
</tr>
<tr>
<td>(S-84)E6, R6*</td>
<td>+(-7.0)</td>
</tr>
</tbody>
</table>

* The donor was W3747. F+(lac). The selective medium was EM agar containing 1.0% lactose and 0.1% L-asparagine. The R factors used are described in Materials and Methods.

† The numbers in parentheses indicate the logarithm of the transmission frequency of the R factor.

<table>
<thead>
<tr>
<th>Recipient in cross</th>
<th>Transmission of F(lac) factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S-84)E, R44*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E, R41*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E, R4*</td>
<td>—</td>
</tr>
<tr>
<td>(S-84)E, R6*</td>
<td>+(-7.0)</td>
</tr>
<tr>
<td>(S-84)E, R6*</td>
<td>+(-7.0)</td>
</tr>
</tbody>
</table>

were F+(lac).R- when selected by the lac marker (Table 2, experiment 1).

As indicated in Table 2 (experiment 3), the R factor of *S. newington* (S-84). R31*. F- and (S-84)E, R44*. F- was still nontransferable by conjugation, as reported previously (Harada et al., 1963). The R factor of *E. coli* W1177 F+(lac). R31* or F+(lac). R44* was transferable by conjugation, whereas the R factor of F-.R* was still nontransferable by conjugation. When *E. coli* W1367. Hfr.SMr was used as a recipient strain, the transmission frequency of F(lac) or R factor was less than \( 10^{-8.4} \) (Table 2, experiment 2).

**Relation between chromosomal transfer and R factor infection.** In a cross of *E. coli* W1177 F+(lac). R* with *E. coli* W4574 F-, 100% of the conjugants selected on TC or lac plates were F+(lac). R*.pro*, and their transmission frequency was \( 10^{5.2} \) or \( 10^{4.3} \), respectively. When selected on EMG agar plates, 67.3% of the pro+ recombinants were F-.R- and 32.7% were F+.R+. R*; the recombination frequency was \( 10^{-6.0} \) (Table 3, experiment 1). In a cross of *E. coli* W1177 F+(lac). R* with *E. coli* 58-161 F-, 100% of the conjugants selected on TC plates were F+(lac). R* (TC).M*, and their transmission frequency was \( 10^{5.8} \). When selected on EMG lac agar plates, 99% of M* recombinants were F+(lac). R* and 1% were F-.R*, a recombination frequency of \( 10^{4.2} \) (Table 3, experiment 1). When *E. coli* W1177 F-.R* was used as a
TABLE 2. Acquisition of transmissibility of R factor of the R\textsuperscript{+} transductants by infection with F factor

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Cross</th>
<th>Donor</th>
<th>Recipient</th>
<th>Selected on\textsuperscript{a}</th>
<th>Transmission of R or F(lac) factor\textsuperscript{b}</th>
<th>Markers of conjugant \textsuperscript{a}</th>
</tr>
</thead>
</table>
| 1        | (S-84)E\textsubscript{2}.R\textsubscript{1}\textsuperscript{+}  
F\textsuperscript{+(lac)} | E. coli W1177  
F\textsuperscript{−}.SM\textsuperscript{+}.lac\textsuperscript{−} | TC.SM, BTB-lactose agar  
Lac.SM,EM(TLB\textsubscript{2}) | F\textsuperscript{+(lac)}, R\textsubscript{44}\textsuperscript{+} (98%)  
F\textsuperscript{−}(lac), R\textsubscript{44}\textsuperscript{+} (2%)  
F\textsuperscript{−}(lac), R\textsubscript{44}\textsuperscript{−} (100%) | F\textsuperscript{+(lac)}, R\textsubscript{44}\textsuperscript{+} (100%) |
| (S-84)E\textsubscript{1}.R\textsubscript{0}\textsuperscript{+}  
F\textsuperscript{+(lac)} | E. coli W1177  
F\textsuperscript{−}.SM\textsuperscript{+}.lac\textsuperscript{−} | TC.SM, BTB-lactose agar  
Lac.SM,EM(TLB\textsubscript{2}) | F\textsuperscript{−}(lac), R\textsubscript{44}\textsuperscript{−} (100%) | |
| 2        | (S-84)E\textsubscript{2}.R\textsubscript{1}\textsuperscript{+}  
F\textsuperscript{+(lac)} | E. coli W1367  
Hfr.SM\textsuperscript{−} | TC.SM, BTB-lactose agar  
TC.SM, BTB-lactose agar | − (<−9.4)  
− (<−9.4) | |
| (S-84)E\textsubscript{1}.R\textsubscript{0}\textsuperscript{+}  
F\textsuperscript{+(lac)} | E. coli W1367  
Hfr.SM\textsuperscript{−} | TC.SM, BTB-lactose agar  
TC.SM, BTB-lactose agar | − (<−9.4)  
− (<−9.4) | |
| 3        | (S-84)E\textsubscript{2}.R\textsubscript{1}\textsuperscript{−}  
F\textsuperscript{−} | E. coli W1177  
F\textsuperscript{−}.SM\textsuperscript{−} | TC.SM, BTB-lactose agar  
TC.SM, BTB-lactose agar | − (<−9.4)  
− (<−9.4) | |
| (S-84)E\textsubscript{1}.R\textsubscript{0}\textsuperscript{−}F\textsuperscript{−} | E. coli W1177  
F\textsuperscript{−}.SM\textsuperscript{−} | TC.SM, BTB-lactose agar  
TC.SM, BTB-lactose agar | − (<−9.4)  
− (<−9.4) | |

\textsuperscript{a} BTB = bromothymol blue; EM(TLB\textsubscript{2}) = EM agar enriched with threonine, leucine, and vitamin B\textsubscript{12}.

\textsuperscript{b} Numbers in parentheses indicate the logarithm of the transmission frequency of the R or F factor after 2 hr of mixed cultivation; − = a transmission frequency of less than 10\textsuperscript{−4}.

\textsuperscript{c} Numbers in parentheses indicate the percentage of the colonies tested carrying the indicated markers.

\textsuperscript{d} Transduced and lysogenized with \textsuperscript{e}E\textsubscript{3.1}, which has no converting ability.

donor, neither TC\textsuperscript{+} conjugants nor M\textsuperscript{+} recombinants developed on TC or EMlac agar plates (Table 3).

As indicated in Table 3 (experiment 1), the transmission frequency of TC in conjunction with F(lac) factor was 10\textsuperscript{−8.9} or 10\textsuperscript{−2.2}, and a recombination frequency of less than 10\textsuperscript{−} times was noted when proline or methionine was used as a selective marker. No relationship existed between the transmission of F(lac).R and chromosomal transfer of proline or methionine. These results indicated that the transmission of otherwise nontransferable R factor with F(lac) factor occurs through conjugation and not by chromosomal transfer. The presence of F\textsuperscript{+(lac)}.R\textsuperscript{+} strains among pro\textsuperscript{+} or M\textsuperscript{+} recombinants indicates that F(lac).R factor was transmitted independently of chromosomal transfer and with a frequency 10\textsuperscript{3} times higher than that of recombination.

As shown in Table 4, the transmission frequency of TC resistance in conjunction with F(lac) factor was 10\textsuperscript{2} to 10\textsuperscript{3} times higher than the frequency of intrinsic chromosomal transfer when recombinants were selected on EMG agar plates (experiment 1) or on EMlac agar plates (experiment 2). When \textit{E. coli} W4574 F\textsuperscript{+(lac)} or 58-161 F\textsuperscript{+} was used as a recipient, the transmission frequency of F(lac).R factor by conjugation was about 10\textsuperscript{3} times lower than that when \textit{E. coli} W4574 F\textsuperscript{−} (experiment 1) or 58-161 F\textsuperscript{−} was used as a recipient. These results indicate that the infective transmission of R factor is consistently accompanied by F(lac) infection, and the transmission frequency of F(lac).R factor was decreased by interference of the F factor present in a recipient strain. This phenomenon was reported in the study on infective transmission of F to F\textsuperscript{+} recipient.

\textit{Joint elimination of F(lac).R factor by treatment with acridine dye.} The mechanism of joint transmission of F(lac).R factor, F\textsuperscript{+(lac)}.R\textsuperscript{+} conjugant to which both factors were simultaneously transferred by conjugation was examined by use of acridine orange. As can be seen in Table 5, the R factors of \textit{Salmonella} (S-84)E\textsubscript{3}. R\textsubscript{44}\textsuperscript{+} and of \textit{E. coli} W1177.F\textsuperscript{−}.R\textsubscript{44}\textsuperscript{+} were not eliminated by treatment with acridine orange,
### Table 3. Relation between the acquisition of transferability of R factor by F(lac) factor and F mating

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Cross</th>
<th>Selected on</th>
<th>Transmission or recombination</th>
<th>Markers of conjugants or recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli W1177 F&lt;sup&gt;+&lt;/sup&gt;(lac).R&lt;sub&gt;R21&lt;/sub&gt;(^{+}) T&lt;sup&gt;-&lt;/sup&gt;.L&lt;sup&gt;-&lt;/sup&gt;.B&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>E. coli W4574 F&lt;sup&gt;-&lt;/sup&gt;.pro&lt;sup&gt;-&lt;/sup&gt;.lac&lt;sup&gt;-&lt;/sup&gt;</td>
<td>TC.pro, EMGGa</td>
<td>F&lt;sup&gt;+&lt;/sup&gt;(lac), R&lt;sub&gt;R21&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;, pro&lt;sup&gt;-&lt;/sup&gt; (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lac.pro, EMAsp</td>
<td>F&lt;sup&gt;+&lt;/sup&gt;(lac), R&lt;sub&gt;R21&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;, pro&lt;sup&gt;-&lt;/sup&gt; (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EMG</td>
<td>F&lt;sup&gt;+&lt;/sup&gt;(lac), R&lt;sub&gt;R21&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;, pro&lt;sup&gt;-&lt;/sup&gt; (32.7%)</td>
</tr>
<tr>
<td>2</td>
<td>E. coli W1177 F&lt;sup&gt;+&lt;/sup&gt;(lac).R&lt;sub&gt;R21&lt;/sub&gt;(^{+}) T&lt;sup&gt;-&lt;/sup&gt;.L&lt;sup&gt;-&lt;/sup&gt;.B&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>E. coli 58-161 F&lt;sup&gt;-&lt;/sup&gt;.M&lt;sup&gt;-&lt;/sup&gt;.lac&lt;sup&gt;+&lt;/sup&gt;</td>
<td>TC.M, EMLacGa</td>
<td>F&lt;sup&gt;+&lt;/sup&gt;(lac), R&lt;sub&gt;R21&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;, M&lt;sup&gt;-&lt;/sup&gt; (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lac, EM</td>
<td>F&lt;sup&gt;+&lt;/sup&gt;(lac), R&lt;sub&gt;R21&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;, M&lt;sup&gt;-&lt;/sup&gt; (99%)</td>
</tr>
</tbody>
</table>

* The R<sub>R21</sub> factor of the donor strains was transferred by mixed cultivation in conjunction with F(lac) factor from the R<sup>+</sup> transductant of Salmonella (S-84)E2 by epsilon phage.

† EMGga = EM agar containing 1.0% glucose and 0.5% sodium glutamate. EMAsp = EM agar containing 0.1% l-asparagine. EMLacGa = EM agar containing 0.5% sodium glutamate and 1.0% lactose instead of glucose.

The numbers in parentheses indicate the logarithm of transmission frequency of the R or F(lac) factor, or recombination frequency after 2 hr of mixed cultivation.

* The markers of conjugants or recombinants indicated above.

* Indicates the donor strains carried R<sub>R21</sub> factor but not F(lac) among the donors described above.

whereas the F(lac).R factor of F<sup>+</sup>(lac).R<sup>+</sup> conjugants, to which both factors were jointly transferred by mixed cultivation, was eliminated together with a high frequency (93.2 to 100%). Strains of F<sup>-</sup>.R<sup>-</sup>.lac<sup>-</sup>, F<sup>-</sup>.R<sup>-</sup>.lac<sup>+</sup>, and F<sup>+</sup>.R<sup>-</sup>.lac<sup>-</sup> appeared as a result of treating E. coli W1177 F<sup>+</sup>(lac).R<sup>+</sup> with acridine dye. This result shows that the elimination of R factor in F<sup>+</sup>(lac).R<sup>+</sup> conjugants was consistently accompanied by the elimination of F factor, although the segregation of lac gene from F(lac) factor was found. The presence of a F<sup>+</sup>.R<sup>-</sup>.lac<sup>-</sup> strain after treatment with acridine dye indicated also the segregation of lac gene from F(lac) factor. The F(lac) and R factors in [F<sup>+</sup>(lac)].R<sup>+</sup> conjugant to which F(lac) factor was transferred to the R<sup>+</sup> strain by mixed cultivation were eliminated separately by acridine orange; joint transmission of both F and R factors was not found so far as tested, indicating that both F(lac) and R factors were present separately in the host bacteria.

**Joint transmission of F(lac).R factor.** To understand the state of F(lac).R factor in a host bacterium, the joint transmission of F(lac).R factor was studied by the blender technique. The F(lac).R factor was transferred from Salmonella (S-84)E2.F<sup>+</sup>(lac).R<sup>+</sup> to E. coli W1177 F<sup>-</sup> by mixed cultivation. In a cross of W1177 F<sup>+</sup>.lac<sup>-</sup>.R<sup>+</sup> with E. coli W4861 F<sup>-</sup>, the F(lac).R factor is transmitted to the recipient cells by mixed cultivation for about 9 min. Detailed results will be reported elsewhere. The transmission frequency of F(lac).R factor by mixed cultivation for 10 min was 10<sup>-1.7</sup> to 10<sup>-3.2</sup> as shown by selection on TC, lac, or (TC).lac plate. The F(lac) and R factors were transferred as a single unit; however, attempts to detect the transfer of either F(lac) or R factor as a separate unit after blender treatment were unsuccessful (Table 6). This fact strongly suggests that the acquisition of transmissibility of the otherwise nontransmissible R factor by F(lac) is attributable to the F(lac).R factor which was newly formed by the combination of the R and F(lac) factors present together in a host bacterium.

**Transduction of F(lac).R factor with phage**
Table 4. Decrease of the transmission frequency of R factor in conjunction with F(lac) factor to F+ recipients

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Recipient in cross</th>
<th>Selected on</th>
<th>Transmission or recombination</th>
<th>Markers of conjugants or recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli W5474</td>
<td>TC.pro, EMGGa</td>
<td>F+(lac), R(_{51}), pro-</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td>F(-).pro(-).lac-</td>
<td>Lac.pro, EMAsp</td>
<td>F+(lac), R(_{51}), pro-</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EMG</td>
<td>F+(lac), R(_{51}), pro-</td>
<td>(32.7%)</td>
</tr>
<tr>
<td></td>
<td>E. coli W5474</td>
<td>TC.pro, EMGGa</td>
<td>F+(lac), R(_{51}), pro-</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td>F+(lac).pro-</td>
<td>EMG</td>
<td>F+(lac), R(_{51}), pro-</td>
<td>(72%)</td>
</tr>
<tr>
<td></td>
<td>lac+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>E. coli 58-161</td>
<td>TC.M, EMLaGa</td>
<td>F+(lac), R(_{51}), M-</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td>F(-).M(-).lac+</td>
<td>Lac, EM</td>
<td>F+(lac), R(_{51})(TC), M+</td>
<td>(99%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F+, R- , M+</td>
<td>(1%)</td>
</tr>
<tr>
<td></td>
<td>E. coli 58-161</td>
<td>TC, M. EMLaGa</td>
<td>F+(lac), R(_{51}), M-</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td>F+(lac).M(-).lac+</td>
<td>Lac, EM</td>
<td>F+(lac), R(_{51}), M+</td>
<td>(96%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F+, F-(lac), R-, M+</td>
<td>(4%)</td>
</tr>
</tbody>
</table>

* The donor in all crosses was E. coli W1177 F+(lac), R\(_{51}\) T\(_{c}\)L\(-\)B\(_{L}\)\(-\). 

b See footnote b of Table 3.

c Numbers in parentheses indicate the logarithm of transmission frequency of the F(lac) or R factor, or of recombination frequency after 2 hr of mixed cultivation.

d See footnote c of Table 2.

e The presence of F factor of E. coli 58-161 F\(+\) used as a recipient was not investigated.

PI\(_{kc}\). Phage PI\(_{kc}\) was grown on E. coli W1177 F\(+\)(lac).R\(_{51}\) or W4574 F\(+\)(lac).R\(_{51}\). When selected on a TC plate, the transmission frequency of TC marker was 10\(^{-2}\); the markers of transductants are shown in Table 7. F\(+\).R\(+\).lac- transductant was not found, and about 9% of the R\(+\) transductants were F\(+\).R\(+\).lac- (experiments 1 and 2). When selected on a lac plate, F\(+\).lac-, R- and F\(+\).lac-, R- transductants were obtained (experiment 2). These results indicate that F and R factors were transferred with PI\(_{kc}\) phage, and the lac gene was consistently segregated from F(lac) factor when selected on a TC or lac plate.

When phage PI\(_{kc}\) was propagated on the donor E. coli W4574-t, F\(+\).R\(+\).lac- or E. coli W4574-t2 F\(+\).R\(+\).lac-\(-\), about 90% of the R\(+\) transductants were F\(+\).R\(+\).lac-, and the transmission frequency of TC marker was 10\(^{-1}\). The joint transmission of F.R factor from F\(+\).R\(+\) conjugants or from F\(+\).R\(+\) transductants with a high frequency indicates the recombination of F(lac) and R factor in a host bacterium and the formation of F(lac).R factor.

DISCUSSION

The R factor of R\(+\) transductants with phage \(\epsilon\) was nontransferable by mixed cultivation and was not eliminated by treatment with acridine dye (Harada et al., 1963). To determine the mechanism involved in the nontransferability of R factor in the R\(+\) transductant obtained with phage \(\epsilon\), Salmonella (S-84)R\(+\) was infected with F(lac) factor. When F(lac) factor was transferred to Salmonella (S-84)R\(+\), the otherwise nontransferable R factor of Salmonella (S-84)R\(+\) acquired transferability by mixed cultivation in conjunction with F(lac) factor. The F(lac).R factor was jointly transferred by conjugation with a high frequency. The conjugants to which F(lac).R factor was jointly transferred by conjugation were still able to transfer their F(lac).R factor
TABLE 5. Joint elimination of F(lac). R factor by treatment with acridine orange

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Complete or segregated elimination of F(lac) and R factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With acridine orange</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R-</td>
</tr>
<tr>
<td>Salmonella (S-84)E2</td>
<td>R21+</td>
<td>0</td>
</tr>
<tr>
<td>[F+(lac)].R21+</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>E. coli W1177</td>
<td>F+(lac).R21+</td>
<td>0</td>
</tr>
<tr>
<td>[F+(lac)].R21+</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>E. coli W4574</td>
<td>F+(lac).R21+</td>
<td>0</td>
</tr>
</tbody>
</table>

* F+(lac).R21+ strains of E. coli were F+(lac).R21+ conjugants, to which F(lac) factor was transferred by conjugation. Numbers in the table indicate the percentage of strains carrying the indicated markers among the total colonies (300 to 500) tested.
* Salmonella (S-84)E2 R21+ was R+ transductant with e34-1 phage, and its R factor was nontransferable by conjugation.
* The F(lac) factor was transferred to Salmonella (S-84)E2 R21+ and E. coli W1177 F+(lac).R21+ by conjugation.
* E. coli W1177 F+(lac).R21+ was R+(TC) conjugant, to which only R(TC) factor was transferred from Salmonella (S-84)E2[F+(lac)].R21+ by conjugation.

TABLE 6. Joint transmission of F(lac). R factor by conjugation

<table>
<thead>
<tr>
<th>Selected on</th>
<th>Transmission time of (F(lac).R)</th>
<th>Transmission frequency (for 10 min)</th>
<th>Conjugant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td></td>
<td>Marker</td>
</tr>
<tr>
<td>TC. ade, EMGGa</td>
<td>81/2</td>
<td>-1.9</td>
<td>F+lac+R+</td>
</tr>
<tr>
<td>Lac. ade, EMAsp</td>
<td>81/2</td>
<td>-1.7</td>
<td>F+lac+R+</td>
</tr>
<tr>
<td>TC. Lac. Ade, EMAsp</td>
<td>83/4</td>
<td>-2.0</td>
<td>F+lac+R+</td>
</tr>
</tbody>
</table>

* The donor was E. coli W1177 F+(lac).R21+T-.L-.B1+; the recipient was E. coli W4861 F−pur−.lac−.
* See footnote b of Table 3.
* The numbers indicate the logarithm of transmission frequency by mixed cultivation for 10 min.
* The transferability of F(lac). R factor of F+(lac).R21+ conjugant of E. coli W4861 was tested by mixed cultivation with E. coli W4754 F−.

by cell-to-cell contact. When the results of the blender technique were analyzed, it was found that the F(lac).R factor of F+(lac).R+ conjugants was jointly transferred by conjugation after 8 to 9 min of mixing. Furthermore, the joint elimination of F(lac).R factor of E. coli F+(lac).R+, to which F(lac).R factor was jointly transferred by conjugation, was 93.2 to 100% when treated with acridine dye. In the transductional analysis of F(lac). R factor, the F. R factor was jointly transduced with PlkC phage, but the lac gene was consistently segregated from the
TABLE 7. Joint transduction of newly formed F\(^+(lac)\).R factor with phage P1kc

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Phage(^a)</th>
<th>System</th>
<th>MOI(^b)</th>
<th>Selected on(^c)</th>
<th>Frequency of transduction(^d)</th>
<th>Markers of transductant(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Donor</td>
<td>Recipient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>P1kc</td>
<td>W1177</td>
<td>W1177</td>
<td>TC, BTB-lactose  agar</td>
<td>-5.0 6/58</td>
<td>52/58</td>
</tr>
<tr>
<td></td>
<td>(W1177)</td>
<td>F(^+)(lac)</td>
<td>F(^-).lac(^-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>P1kc</td>
<td>W4574</td>
<td>W4574</td>
<td>TC, BTB-lactose  agar</td>
<td>-5.0 31/374</td>
<td>343/374</td>
</tr>
<tr>
<td></td>
<td>(W4574)</td>
<td>F(^+)(lac)</td>
<td>F(^-).lac(^-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>P1kc</td>
<td>W4574(\uparrow),&lt;sub&gt;t1&lt;/sub&gt;</td>
<td>W4574</td>
<td>TC, BTB-lactose  agar</td>
<td>-5.3 48/49</td>
<td>1/49</td>
</tr>
<tr>
<td></td>
<td>(W4574-&lt;sub&gt;t1&lt;/sub&gt;)</td>
<td>F(^+), R(_{NI}), Lac(^-)</td>
<td>F(^-).lac(^-), W1177</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>P1kc</td>
<td>W4574(\uparrow),&lt;sub&gt;t2&lt;/sub&gt;</td>
<td>W4574</td>
<td>TC, BTB-lactose  agar</td>
<td>-5.1 47/51</td>
<td>4/51</td>
</tr>
<tr>
<td></td>
<td>(W4574-&lt;sub&gt;t2&lt;/sub&gt;)</td>
<td>F(^+), R(_{NI}), lac(^-)</td>
<td>F(^-).lac(^-), W1177</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The propagating strain of phage is shown in parentheses.

\(^b\) Multiplicity of infection.

\(^c\) BTB = bromothymol blue; EMAsp = EM agar containing 0.1% l-asparagin.

\(^d\) Numbers indicate the logarithm of transduction frequency.

\(^e\) Denominator indicates the total number of colonies tested. None of the colonies tested had F\(^+\)R\(^+\)-lac\(^+\) or F\(^-\)R\(^+\)lac\(^+\) markers.

\(^f\) W4574-<sub>t1</sub> and W4574-<sub>t2</sub> indicate the F\(^+\).R\(^+\) transductant of E. coli W4574 obtained in experiments 2 and 3, respectively. The F\((lac)\).R\(_{NI}\) factor of E. coli W1177 and W4574 used as donors was transferred from Salmonella (S-84)E\(_2\)[F\((lac)\).R\(_{NI}\)](TC) by mixed cultivation, to which F\((lac)\) factor was transferred to R\(^+\)(TC) transductant of Salmonella (S-84)E\(_2\) with episom phage.

F\((lac)\).R factor when selected on a TC plate. From these results, it is concluded that the acquisition of transferability of the otherwise nontransmissible R factor of the R\(^+\) transductant of Salmonella (S-84) with \(\phi\) phage is mainly caused by the formation of the recombinant F\((lac)\).R factor when F\((lac)\) factor was transferred to Salmonella (S-84)R\(^+\) upon conjugation. The elimination of F\((lac)\).R factor at a high frequency in the presence of acridine dye is attributed to the fact that the newly formed F\((lac)\).R factor is in the extrachromosomal state.

When Salmonella (S-84) F\(^+\)(lac).R\(^+\), obtained by infection with F\((lac)\) factor to the R\(^+\) transductant with phase \(\phi\), 78.7% of the total colonies, treated with acridine dye, were F\(^-\).lac\(^-\).R\(^+\), indicating that usually the two factors existed separately in a host bacterium (Table 8). Consequently, there are two possibilities to consider for the existence of both factors in a host bacterium, Salmonella (S-84) F\(^+\)(lac).R\(^+\): the formation of recombinant F\((lac)\).R factor and the separate existence of both factors. The recombinant F\((lac)\).R factor was transferred with a high frequency (10\(^{-2.9}\) to 10\(^{-3.3}\)) by mixed cultivation, and 100% of the conjugants acquired F\((lac)\).R factor without the transfer of intrinsic chromosomal markers, such as pro, M. This is accounted for by a transmission frequency of F\((lac)\).R factor 10\(^{6}\) to 10\(^{4}\) times higher than the recombination frequency of chromosomal marker of pro or M. As shown in Tables 2 and 4, the transmission rate of F\((lac)\).R factor was decreased when the recipient strains carried the F factor (or Hfr). This decrease is due to the interference of F factor (Hirota, 1959; Scaife and Gross, 1962; Harrison, 1963), and it shows that transmission of the R of F\((lac)\).R factor is conducted consistently along with the F\((lac)\) factor.

It is known that the factors not transmitted singly by colicinogenic S. typhimurium LT2 strains, namely, colE2 and colK, and the poorly
transmissible colE1, were transmitted, with the transmissible factor concerned, to many acceptor bacteria by doubly colicinogenic donor strains carrying colB or colB as well as colE2, colK, or colE1. When the nontransmissible col factor acquired transferability by double infection of transmissible col factor, both factors were transferred separately by a short period of mixed culture (Ozeki, Stocker, and Smith, 1962). This fact suggests the existence of another possibility for the acquisition of transferability of otherwise nontransmissible R(TC) factor, with F(lac) factor involved, rather than the formation of recombinant F(lac).R. However, this has not been found in the case when transferability of otherwise nontransferable R factor described herein was acquired.

The detailed mechanism of recombination of F(lac) and R factor and the genetic structure of F(lac).R factor have not been clearly understood. However, it is suggested that the lac gene of F(lac).R factor will locate at the terminal end of this factor, because the F.R factor is obtained from the F(lac).R factor upon treatment with acridine dye or transduction with P1ke phage.

Acknowledgments

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Literature Cited


