Drug Resistance of Enteric Bacteria

VI. Introduction of Bacteriophage P1CM into *Salmonella typhi* and Formation of P1dCM and F-CM Elements

EIKO KONDO AND SUSUMU MITSUHASHI

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

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ABSTRACT

KONDO, EIKO (Gunma University, Maebashi, Japan), AND SUSUMU MITSUHASHI. Drug resistance of enteric bacteria. VI. Introduction of bacteriophage P1CM into *Salmonella typhi* and formation of P1dCM and F-CM elements. J. Bacteriol. 91: 1787-1794. 1966.—Bacteriophage P1CM was introduced into *Salmonella typhi* by means of both phage infection and conjugation with *Escherichia coli* F⁺ lysogenic for the phage. Upon incubation with a P1CM phage lysate, *S. typhi* and *S. abony* yield CM^r cells which are lysogenic for P1CM, but *S. typhimurium* LT2 does not. The P1CM phage is adsorbed slightly to *S. typhi*, but no infectious centers are formed when the phage is plated on this strain. Tests on P1CM-adsorbing capacity of the *S. typhi* P1CM⁺ strain and on plaque formation and transduction ability of the recovered phage from this strain indicated that the cell and the phage population did not have any special advantage over the original cell and phage population. Conjugation of *S. typhi* with *E. coli* F⁺ carrying P1CM⁺ gave three types of *S. typhi* CM^r clones: those which carry the whole P1CM phage, those with the P1dCM element, and those with nontransferable CM^r. The second type has the F factor and is sensitive to f phages in spite of its typical behavior, serologically and biochemically, as *S. typhi*. It can donate the P1dCM and F⁺ characters to *E. coli* F[−] or F[−]/P1 strains. As a consequence of conjugation with the *E. coli* F⁺ strain, the CM^r character of the third type of *S. typhi*, the nontransferable CM^r element, acquired conjugational transferability, owing to the formation of the element, F-CM. This element can be transferred to an *E. coli* F[−] strain at a very high frequency (ca. 10⁶). Both the F and CM^r determinants are jointly transduced with P1 phage and are jointly eliminated by acridine dye treatment.

In a previous paper (16), bacteriophage P1CM was reported to carry all the pertinent phage activities of P1, as well as the CM^r character. P1CM multiplies by lytic growth, forming plaques similar to that of normal P1. Transduction of CM^r to recipient cells occurs following lysogenization.

It is known that phage P1 grows on *Shigella* (*dysenteriae*) and *Escherichia coli* (B, C, and K) strains (5). Since *Salmonella typhi* was thought to be unaffected by P1 or P1CM infection, it was used as a recipient for testing transfer by conjugation of the CM^r portion of P1CM. *S. typhi* CM^r clones were obtained, however, as a result of mixed incubation, not only with the P1CM-carrying strain, but also with the cell-free lysate of P1CM.

This paper deals with the introduction of P1CM into *S. typhi* by phage infection and through

conjugation with *E. coli* carrying the F factor and P1CM prophage. Whereas infection of *S. typhi* by P1CM produced only *S. typhi* lysogenic for P1CM, conjugation with *E. coli* F⁺ P1CM⁺ resulted in *S. typhi* clones carrying various P1CM derivatives which were different in P1 function, i.e., complete P1CM, defective P1CM, or only the CM^r portion.

MATERIALS AND METHODS

Microorganisms. Bacterial strains used are listed in Table 1. *S. dysenteriae* Sh/s was used as the indicator strain for phage P1CM as well as phage P1. *S. typhi* H901 and *S. abony* 74 are standard strains of *Salmonella*. Resistant strains (P1) were prepared as needed.

Bacteriophages used were P1CM, f1 [isolated by Loeb (18)], and f2 [isolated by Loeb and Zinder (19)]. Phage lysates were prepared by the agar layer method (31) and sterilized with chloroform. P1CM

TABLE 1. *Bacterial strains used*

Strain	Relevant characters
<i>Shigella</i>	
<i>S. dysenteriae</i> Sh/s.....	SM ^r
<i>Salmonella</i>	
<i>S. abony</i> 74....	<i>lac</i> ⁻
<i>S. typhimurium</i> LT2.....	<i>lac</i> ⁻
<i>S. typhi</i> H 901. St-1 and St-22.	<i>lac</i> ⁻
St-3.....	F ⁺ , P1dCM ⁺ , <i>lac</i> ⁻ (by cross of <i>S. typhi</i> H 901 with <i>E. coli</i> Tr 30-5)
ML 1154.....	<i>lac</i> ⁻ , CM ^r (by cross of <i>S. typhi</i> H 901 with <i>E. coli</i> Tr 30-5)
	F ⁻ , <i>lac</i> ⁻ , P1CM ⁺ (by transducing <i>S. typhi</i> H 901 with P1CM)
<i>Escherichia coli</i>	
K-12 F ⁺ wild..	F ⁺ , prototroph
K-12 F ⁻ wild..	F ⁻ , prototroph (by elimination of F with acridine orange treatment of F ⁺ wild strain)
K-12 W3110....	F ⁻ , <i>Lp</i> ⁻
K-12 W1895....	Hfr, <i>met</i> ⁻
K-12 W3630....	♀ _s , <i>mal</i> ⁻
K-12 W4574....	F ⁻ , <i>pro</i> ⁻ , <i>lac</i> ⁻ , <i>ara</i> ⁻ , <i>mtl</i> ⁻ , <i>xyl</i> ⁻ , <i>mal</i> ⁻ , <i>gal</i> ⁻ , SM ^r
K-12 W2984....	F ⁻ , <i>pro</i> ⁻
K-12 58-161 F ⁻	F ⁻ , <i>met</i> ⁻
K-12 PA200....	F ⁻ , <i>thr</i> ⁻ , <i>leu</i> ⁻ , <i>thi</i> ⁻ , <i>his</i> ⁻ , <i>arg</i> ⁻ , <i>lac</i> ⁻ , <i>ara</i> ⁻ , <i>mtl</i> ⁻ , <i>xyl</i> ⁻ , <i>mal</i> ⁻ , <i>gal</i> ⁻ , <i>Lp</i> ^r , SM ^r
Tr 30-5.....	F ⁺ , P1CM ⁺ (by transducing <i>E. coli</i> K-12 F ⁺ with P1CM)
Tr 62-3.....	F ⁻ , <i>Lp</i> ⁻ , P1CM ⁺ (by transducing <i>E. coli</i> K-12 W3110 with P1CM propagated on the strain)
K57.....	(F-CM) ⁺ , prototroph (by cross of <i>E. coli</i> K-12 F ⁺ wild type with St-3)

lysates were usually prepared by using P1CM phage (10⁶ to 10⁷ per milliliter) obtained by ultraviolet (UV) induction of *E. coli* Tr 62-3 (F⁻ P1CM⁺; 16), with *E. coli* K-12 W3110 as the propagating strain.

The following notations are used: *lac*, lactose; *ara*, arabinose; *mtl*, mannitol; *xyl*, xylose; *mal*, maltose; *gal*, galactose; *met*, methionine; *pro*, proline; *thr*, threonine; *leu*, leucine; *thi*, thiamine; *his*, histidine; *arg*, arginine; *Lp*, chromosomal locus of prophage λ; CM, chloramphenicol; TC, tetracycline; SM, streptomycin; +, fermenting a sugar, not requiring a nutrient or carrying a genetic element; -, non-fermenting, requiring, or not carrying a genetic element; r, resistant; s, sensitive; i, immune.

Media and stock solutions of drugs were prepared according to methods reported previously (16).

Assay of phage and bacteria, and the transduction procedures were the same as described in a previous paper (16). All incubations were done at 37 C.

P1 lysogeny test. A loopful of L-broth culture was spotted onto L-broth agar containing SM (200 µg/ml), seeded previously with *S. dysenteriae* Sh/s (6). Strains lysogenic for P1 or P1CM give a similar positive response in this test.

P1 immunity test. P1 lysates (ca. 10⁸ phage per milliliter) were spotted onto a bacterial lawn streaked on eosin-methylene blue (EMB) complete agar without sugar. Strains which did not give lysis with P1, but were lysed by virulent P1, were classified as P1ⁱ.

Anti-P1 phage serum. The same antiserum described in a previous paper was used (16).

Bacterial crosses by mixed cultivation. At various times of incubation, after mixing donor and recipient cultures, suitable samples of the mixture were spread on appropriate selective plates, on which neither of the parent strains could grow alone. The frequency of transfer in a particular cross was determined by dividing the number of colonies which appeared on the selective plate by the number of donor cells in the initial mating mixture.

Sensitivity of bacteria to phage. A loopful of phage stock (ca. 10⁹ phage per milliliter) was spotted onto a bacterial lawn seeded on EMB complete agar without sugar.

Method of treatment with acridine dye. Overnight L-broth cultures of test strains were inoculated into L-broth (pH 7.8) containing acridine dye in various concentrations (12). After overnight incubation, the cells were plated on bromothymol blue-lactose-nutrient agar, and the CM resistance of the colonies which developed on incubation was tested. The test for the presence of the F factor was based on f phage sensitivity after three successive single-colony isolations of the colony.

RESULTS

Infection of *Salmonella* strains by phage P1CM. After incubation of *S. typhi* H901 with a P1CM lysate prepared on an *E. coli* F⁺ *Lp*⁺ host, *S. typhi* H901 clones carrying P1 and resistant to CM could be isolated. Other strains of *Salmonella*, *S. abony* 74 and *S. typhimurium* LT2, were similarly tested, but, as shown in Table 2, only *S. typhi* and *S. abony* could be transduced with P1CM. Transduction frequencies were about 10⁻⁶ to 10⁻⁷ per input phage or about 10⁻⁸ per input cell. P1CM lysates derived from F⁻ *Lp*⁻ strains can transduce the *Salmonella* strains with the same efficiency as that of P1CM lysates derived from the F⁺ *Lp*⁺ strain. This indicates that neither the F factor nor λ phage carried by a donor cell contributes to this system. Moreover, the formation of CM-resistant *Salmonella* colonies could be inhibited completely by prior treatment with anti-P1 phage serum.

TABLE 2. Infection of P1CM phage in *Salmonella* and formation of CM-resistant strains*

Prepn	Plaque-forming units/ml	<i>S. typhi</i>		<i>S. abony</i>		<i>S. typhimurium</i>	
		MOI	No. CM ^r	MOI	No. CM ^r	MOI	No. CM ^r
Lysate prepared by propagating UV-induced P1CM from <i>E. coli</i> Tr 62-3, on <i>E. coli</i> F ⁺	8.4×10^9	0.3	6 (3.6×10^{-7})	0.4	6 (3.6×10^{-7})	0.1	0
Lysate prepared by propagating UV-induced P1CM from <i>E. coli</i> Tr 62-3, on <i>E. coli</i> W3110	1.5×10^{10}	0.5	14 (4.8×10^{-7})	0.3	25 (8.6×10^{-7})	0.2	0
Lysate prepared by propagating P1CM released from <i>E. coli</i> Tr 30-5, on <i>E. coli</i> F ⁺	7.0×10^9	0.3	8 (5.7×10^{-7})	0.1	8 (5.7×10^{-7})	0.1	0

* Each 0.2 ml of a 10^{-1} dilution of phage lysates, and each 1.8 ml of bacterial culture (*S. typhi*, 3.1×10^8 cells per milliliter; *S. abony*, 6.6×10^8 ; *S. typhimurium* LT2, 9.6×10^8) containing 3×10^{-3} M CaCl₂, were mixed and incubated for 90 min; 0.2 ml of the mixture was spread on Heart Infusion Agar containing CM (25 µg/ml), and the number of colonies which developed were counted after 48 hr of incubation. Figures in parentheses show the transduction frequency expressed as the number of CM^r colonies per number of input phage. MOI, multiplicity of infection.

The CM-resistant colonies of *S. typhi* and *S. abony* were purified by four successive single-colony isolations, and were tested for P1 lysogeny. They were all found to be lysogenic. The spontaneous loss of the CM^r character was examined in five CM^r clones of each *Salmonella* strain. Tests on 200 to 600 colonies of each clone showed that they were all CM^r. It was found, furthermore, that P1CM was very stable in these clones and was not lost on repeated subculturing or on aging.

These results indicate that some *Salmonella* strains are able to be infected with P1CM phage and that they can give rise to stable phage carriers, just as in lysogeny.

Transduction frequencies by P1CM are usually more than 0.5 when *E. coli* strains are the recipients. In contrast, the frequency of 10^{-6} to 10^{-7} observed in *Salmonella* strains is very low. It seemed useful, therefore, to measure the efficiency of adsorption of P1CM. Phage was mixed with bacteria (ratio of about 1:10) in the presence of CaCl₂ (2.5×10^{-3} M), and the mixture was allowed to stand for 20 min at 37 C. The mixture was then diluted with cold fresh L-broth, and was plated with *S. dysenteriae* Sh/s on L-broth agar containing SM (200 µg/ml) for plaque formation of unadsorbed phage. From 15 to 25% of the added P1CM was adsorbed to *S. typhi* (95 to 98% was adsorbed to *E. coli*). Though its rate is very low, compared with *E. coli*, *S. typhi* adsorbs significant numbers of phage P1CM particles. However, *S. typhi* fails to show plaque

formation with P1CM, even when very large numbers of phage particles were used.

To test whether phage infection of *S. typhi* is due to a host-range mutant, plaque formation and transduction frequency were assayed on *S. typhi* H901 by use of P1CM recovered from the *S. typhi* P1CM⁺ strain, ML 1154. No plaques were produced when the phage lysate was plated on *S. typhi* and, in addition, this phage transduced *S. typhi* to CM^r at a frequency similar to the original P1CM derived from *E. coli*. Therefore, the infection of P1CM in *Salmonella* is not explainable by a special phage mutant, and, after the infection of *Salmonella*, the phage does not exhibit a host-induced modification in host range.

To determine whether the phenomenon was caused by a P1CM mutant with an increased rate of adsorption for the *Salmonella* strain, adsorption efficiency of phage released by *Salmonella* was measured on ML 1154. In general, lysogenized strains show the same capacity to adsorb the lysogenic phage as do the nonlysogenized original strains. It was observed that about 80% of input P1 or P1CM remained unadsorbed, as shown initially for the original *S. typhi* H901 strain. In the case of *E. coli* K-12 P1CM⁺, more than 95% of input P1 or P1CM was adsorbed to the cells.

These results indicate that the infection of *S. typhi* by P1CM is not due to a special mutant of *Salmonella* which has a higher capacity to adsorb phage P1CM.

Formation of F-CM element. Upon mixed

incubation of *S. typhi* with cells of *E. coli* Tr 30-5 (F^+ P1CM⁺), *S. typhi* CM^r cells were obtained. In the course of purification, it was observed that some *S. typhi* CM^r clones gave reddish colonies on EMB medium without sugar; such reddening was lost upon subculturing, as reported by Zinder (33).

Among 22 clones of *S. typhi* CM^r, 7 clones were phage producers, as might be obtained from infection by P1CM phage, but 15 clones did not release phage. Among these 15 clones, 13 were incapable of transferring the CM^r character to *E. coli* W4574/P1 and to W2984/P1, even after 6 hr of incubation. One of these 13 clones, *S. typhi* CM^r (St-3), was subjected to further investigation of its ability to transfer the CM^r character to the following recipients; *E. coli* K-12 F^+ , W1895 Hfr, W4574 F^- , W3630 φ_3 (30), 58-161 F^- , and W2984 F^- . It was found that only *E. coli* F^+ became CM^r after mixed cultivation with St-3. CM^r clones appeared at a very low frequency, one to two clones per 10^8 parent cells after 48 hr of incubation. One of the *E. coli* F^+ CM^r isolates thus obtained was designated as *E. coli* K57. *E. coli* K57 was able to transfer its CM^r character at extremely high frequency (ca. 10^0) and to produce *pro*⁺ recombinants at a frequency of 10^{-5} , in mixed cultivation with *E. coli* W4574. The transfer frequency of the CM^r character was comparable to that of the F factor (17), though the transfer frequency of R factors is usually found to be 10^{-3} to 10^{-4} under our experimental conditions. The transfer of genetic markers other than CM^r was also examined (Table 3). These results indicate that the CM^r character is transferred in extremely high frequency, independent of chromosomal transfer, and that CM^r recipients are also all F^+ , as shown by their sensitivity to f phages.

To elucidate the mechanisms of joint transfer of F and CM^r characters at high frequency, two experiments were performed, one involving transduction with phage P1 and the other involving treatment with acridine.

CM^r transductants of *E. coli* K-12 W3110 and PA 200 were obtained by phage P1 grown on *E. coli* K57 at a multiplicity of infection of 0.2, and they were tested for f phage sensitivity. It was found that 92.5% of these were F^+ (Table 4). Accordingly, it was concluded that the F factor was transduced jointly with the CM^r character in most cases. Moreover, as shown in Table 5, 90% of *E. coli* K57 cells lost the CM^r character after treatment with acridine orange; these clones were all f phage-resistant. In contrast, CM^r clones were all sensitive to f phage. Wild-type R factors in *E. coli* are eliminated by treatment with acridine

dye (25), but the frequency (less than a few per cent) is extremely low compared with that of F factor, which is nearly 100% (12).

From the fact that the CM^r character is consistently associated with the F factor, we conclude that these two characters have become a single unit, forming a complex, the F-CM factor

Isolation of S. typhi strains carrying P1dCM and F, which are susceptible to f phages. Of 15 clones of *S. typhi* CM^r which did not release active P1CM phage, 2 clones (St-1 and St-22) were found to be capable of transferring their CM^r character to *E. coli* F^- at high frequencies (about 10^{-3}).

The CM^r and F determinants in these *S. typhi* strains were transferred to *E. coli* K-12 strains W2984, 58-161 F^- , and wild F^- by mixed cultivation. The *E. coli* strains carrying F and CM^r derived from St-1 were all negative by the P1 lysogeny test, but were immune to phage P1. On the other hand, *E. coli* strains carrying F and CM^r derived from St-22 were all positive by the P1 lysogeny test, producing a small faint plaque on the P1 indicator strain. We then tested whether any P1CM-related particles, i.e., P1 plaque-forming or CM^r-transducing particles, existed in cell-free lysates of these strains before or after UV irradiation. The results (Table 6) revealed that strains carrying F and CM^r derived from St-1 liberated neither P1-plaque-forming nor CM^r-transducing particles, but *E. coli* carrying F and CM^r derived from St-22 spontaneously liberated faint plaque-forming and CM^r-transducing particles. In the original *Salmonella* strains, CM^r transductants did not appear, even when normal P1 phage was added as helper to the transduction mixture. However, as shown in Table 4, upon superinfection with virulent P1 phage, *E. coli* strains carrying F and CM^r derived from St-1 yielded a lysate which gives CM^r-transduction at a frequency of about 10^{-2} , which is considered high frequency of transduction (HFT) according to terminology of Morse et al. (28).

These results indicate that these *E. coli* strains, and possibly St-1, possess a defective P1CM element, or a P1 defective CM element according to the terminology used in certain other transduction systems, for example, λdg (2, 7), P1d1 (22), $\phi 80dt$ (24), and $\epsilon_{18}dR_{28}$ (14).

The results of plaque-center tests (16), shown in the far-right column of Table 6, indicated that the plaque-forming particles released from *E. coli* carrying F and CM^r derived from St-22 possess CM^r-transducing ability. In addition, the CM^r transductants obtained with the lysate from an overnight culture of the *E. coli* strain showed the

TABLE 3. Genetic constitution of recombinants from a cross between *Escherichia coli* W4574 and *E. coli* K57

Incubation time for cross	Selective marker	No. of clones	Character									
			<i>lac</i>	<i>pro</i>	<i>ara</i>	<i>mlt</i>	<i>xyl</i>	<i>mal</i>	<i>gal</i>	<i>CM</i>	<i>SM</i>	f1
<i>hr</i> 1	<i>pro</i>	3	—	+	—	—	—	—	—	r	r	s
		1	—	+	+	—	—	—	—	r	r	s
2	<i>pro</i>	3	—	+	—	—	—	—	—	r	r	s
		5	—	+	+	—	—	—	—	r	r	s
0*	CM	20	—	—	—	—	—	—	—	r	r	s

* The conjugation mixture was plated on selective medium immediately after mixing both donor and recipient.

TABLE 4. Transduction of *CM*^r character with phage P1

<i>Escherichia coli</i> donor strain	<i>E. coli</i> recipient strain	MOI	Transduction frequency per input phage	Phage f1-sensitive clones per no. tested
K57 (F- <i>CM</i>) ⁺	W3110	0.2	2×10^{-6}	37/40
K57 (F- <i>CM</i>) ⁺	PA200	0.2	6×10^{-6}	37/40
W2984 F ⁺ P1d <i>CM</i> _{St-1} ⁺ *.....	PA200	0.1	ca. 10^{-1}	0/39
Wild F ⁺ P1d <i>CM</i> _{St-1} ⁺ *.....	W3110	10^{-5}	3×10^{-2}	0/40
W2984 F ⁺ P1d <i>CM</i> _{St-22} ⁺ *.....	PA200	10^{-3}	ca. 10^{-1}	0/39
wild F ⁺ P1d <i>CM</i> _{St-22} ⁺ *.....	W3110	10^{-5}	9×10^{-3}	0/40

* These are *E. coli* strains carrying F and *CM*^r derived from St-1 and St-2.

TABLE 5. Elimination of *CM*^r character by treatment with acridine orange

Strain	Type of <i>CM</i> ^r	Concn of acridine orange	
		100 µg/ml	0
<i>Escherichia coli</i> K57.....	F- <i>CM</i>	99/110 (90%)	0/280
<i>Salmonella typhi</i> St-1.....	P1d <i>CM</i>	0/687	0/180
<i>S. typhi</i> St-22.....	P1d <i>CM</i>	0/309	0/360
<i>S. typhi</i> St-3.....	Nontransmissible <i>CM</i> ^r	7/471 (1.8%)	0/420
<i>E. coli</i> F ⁺ R ₁₄ ⁺	R factor	1/393 (0.3%)	0/326

same small faint plaque in the P1 lysogeny test. This particle is considered to be slightly defective in P1 phage function, because its plaque is so small and faint. Also, an *E. coli* strain carrying this particle shows less immunity to superinfection by P1, as evidenced by the fairly high titer of P1 lysates (about 10^9 per milliliter) obtained from these strains after superinfection.

Thus, this particle derived from St-22 is also a defective P1*CM*, although the degree of its defectiveness is less than that of P1d*CM* in St-1. These elements are designated as P1d*CM*_{St-22} and P1d*CM*_{St-1}, respectively.

It was also shown that the *CM*^r character in St-1 and St-22, and in *E. coli* strains carrying the F and *CM*^r characters which were derived from

the *S. typhi* strains, could be transferred to either *E. coli* F⁻ W4574 or W4574/P1 at a high frequency (Table 7). However, although *CM*^r recipients are not all F⁺, they are all P1ⁱ. It was further observed that acridine dye treatment failed to eliminate the *CM*^r character of both *S. typhi* strains, St-1 and St-22 (Table 5); the *CM*^r transductants were all resistant to f1 phage (Table 4), being different from those of *E. coli* K57. These facts suggest that the *CM*^r and F⁺ determinants in St-1 and St-22 are not linked to each other, and that they probably exist separately as different elements. St-1 and St-22 themselves were susceptible to f phages, and f phages produce plaques on these strains with the same efficiency of plating as on the *E. coli* male strains.

TABLE 6. *P1CM*-related particle in cell-free supernatant fluid of culture*

Strain	No. of CM ^r transductants			Plaque formation	No. of CM ^r per no. of plaques tested
	Overnight L-broth culture	UV-induced culture	UV-induced culture + helper P1		
St-1.....	0	0	0	—	
St-22.....	0	0	0	—	
<i>E. coli</i> (wild) †.....	0	0	0	—	
F ⁺ P1dCM _{St-1} ⁺					
<i>E. coli</i> (wild) †.....					
F ⁺ P1dCM _{St-22} ⁺	5 × 10 ⁴	2 × 10 ⁴	2 × 10 ⁴	+	87/92

* An overnight L-broth culture was centrifuged. The supernatant fluid was used as the "overnight culture"; the sedimented cells were suspended in saline, and were subjected to UV induction (16). The supernatant fluid, after incubation for 90 min, was used as the "UV-induced culture." The transduction mixture contained 0.1 ml of lysate after chloroform treatment, 0.5 ml of an *E. coli* K-12 F⁺ (wild) culture containing 5 × 10⁸ M CaCl₂ (5 × 10⁸ cells per milliliter) and 0.1 ml of L broth or normal P1 lysate (7 × 10⁸ cells per milliliter) as a helper. After 20 min of incubation, 0.2 ml of the mixture was spread on L broth-agar containing CM (25 µg/ml). The results are expressed as the number of transductants per milliliter of lysate added.

† These are the same strains as used in Table 4.

TABLE 7. *Transfer of CM^r character by conjugation**

Donor	<i>E. coli</i> recipient	No. of CM ^r recipient	Transfer frequency	Phage f1-sensitive clones per no. tested	P1-immune clones per no. tested
<i>Salmonella typhi</i> St-1.....	W4574	9 × 10 ²	10 ⁻⁵	15/19	19/19
	W4574/P1	1.7 × 10 ³	10 ⁻⁵		
<i>Escherichia coli</i> (wild) F ⁺ P1dCM _{St-1} ⁺ †.....	W4574	3.7 × 10 ⁶	10 ⁻²	19/20	20/20
	W4574/P1	5.0 × 10 ⁶	10 ⁻²		
<i>S. typhi</i> St-22.....	W4574	1.9 × 10 ⁶	10 ⁻³		
	W4574/P1	5.2 × 10 ⁶	10 ⁻³		
<i>E. coli</i> (wild) F ⁺ P1dCM _{St-22} ⁺ †....	W4574	1.6 × 10 ⁶	10 ⁻²		
	W4574/P1	9.8 × 10 ⁶	10 ⁻²		

* Equal volumes of L-broth overnight culture (2 × 10⁸ to 5 × 10⁸ cells per milliliter) of donor and recipient strains were mixed and incubated for 2 hr; 0.1 ml of appropriate dilutions were plated on bromothymol blue-lactose-nutrient agar containing CM (25 µg/ml) and SM (200 µg/ml).

† These are the same strains as used in Table 4.

S. typhi strains, i.e., St-1, St-22, and St-3, exhibited the same biochemical and serological properties as *S. typhi* H901.

DISCUSSION

In *E. coli* K-12 strains it has been established that male strains, F⁺, F', or Hfr, can donate their chromosomal characters and cytoplasmic elements to other bacterial cells by conjugation. R factors are genetic elements which resemble the F factor in many respects, although positive evidence for the integration of R factors in a host chromosome has not yet been shown. R factors are mutually transmissible to a wide range of strains belonging to the *Enterobacteriaceae* (10) and *Vibrio* (4).

The results presented in this paper revealed

that phage P1CM infected certain cells of *S. typhi* and established a phage-host relationship analogous to lysogeny. However, the adsorption efficiency of P1CM to *S. typhi* is fairly low. Also, the P1CM released from *S. typhi* P1CM⁺ failed to show infectious centers on *S. typhi* or an increased ability to transduce the CM^r character to *S. typhi* over that of original P1CM. Therefore, the infection of P1CM on *S. typhi* is probably not due to a host-range mutation (20) nor to a host-induced modification of the phage (21).

Moreover, since the *S. typhi* P1CM⁺ strain ML 1154 shows no increased capacity to adsorb P1CM over that of the original *S. typhi* strain, the infection by P1CM of *S. typhi* is not due to a special bacterial mutation which has a higher capacity to adsorb the phage. That *S. typhi* re-

ceives the P1CM phage genome may be considered to be analogous to the ability of this organism to mate with *E. coli*. The fact that *S. typhi* P1CM⁺ does not show a high capacity to adsorb the phage is similar to the situation in which *S. typhi* strains exhibit a low but homogeneous ability of its population to mate initially with *E. coli* Hfr donors (13). In *S. typhimurium* LT7 *mut*⁺, it was demonstrated that fertile recipients are present in the majority of a sterile population (26). *S. typhimurium* LT2 was found to be infertile, and it mated only infrequently. In *S. abony*, some Hfr strains were isolated from the clones which had become F⁺ by receiving the F factor from *E. coli* (23). The infection of P1CM in *Salmonella* strains was found in *S. typhi* and *S. abony*, but not in *S. typhimurium* LT2.

When *E. coli* F⁺ P1CM⁺ was subjected to conjugation with *S. typhi* H901, three types of *S. typhi* CM^r clones were obtained: clones carrying P1CM phage, clones carrying P1-defective CM elements, and clones carrying only nontransmissible CM^r. The second type was found to carry simultaneously the F factor, but the failure of the CM^r element to be transduced jointly with the F character or to be eliminated together by treatment with acridine dye indicated that these two determinants exist separately. One of the third type of *S. typhi* CM^r clones, St-3, showed about a 2% loss of its CM^r character upon treatment with acridine, and its CM^r character was not transferred by conjugation. However, the CM^r character in St-3 acquired the ability to be transferred by conjugation when the strain was crossed with an *E. coli* F⁺ strain. From the experimental results of joint transduction or joint elimination with acridine treatment, it was concluded that the CM^r character had been associated with the F factor, forming an F-CM factor, thereby gaining transferability. The proposed genetic element, the F-CM factor, is a kind of F' (1, 11), though the proposed mechanism for the origination of the usual F' may not apply in the case of F-CM. The ordinary F' strains are derived from integrated F factors, and they carry the chromosomal character(s) which may be adjacent to the F factor. In the case of F-CM, it is likely that the association of both determinants took place in the cytoplasmic state, because the CM^r character in St-3 is presumably in the cytoplasmic state as indicated by the results of acridine dye treatment.

Since the CM^r character in St-3 could be associated with transferability in F⁺ strains but not in Hfr or F⁻, it seems that there are three steps in the acquisition of this transferability: infection of the F factor from the recipient to donor, an association of the F factor with the CM^r determi-

nant in the donor cell, and transfer of the formed F-CM element to the recipient.

Two *S. typhi* P1dCM⁺ strains were isolated which carry the F determinant. It remains obscure as to whether the F factor has any correlation with the carrying of P1dCM by these strains. An experiment to determine whether the CM^r characters in the third type of *S. typhi* CM^r clones are present as a form of P1dCM is impossible at present because of technical limitations.

As a consequence of conjugation between *S. typhi* and *E. coli* F⁺ lysogenic for P1CM, *S. typhi* strains carrying P1dCM elements were obtained. Two P1dCM elements were found to vary in their P1 functions, as noted by the difference in their ability to initiate maturation and interference with the function of normal P1 phage. The diversity of the phage-specific properties of various P1dl elements has been reported by Luria et al. (22).

By means of conjugal transfer from St-22 to *E. coli*, the P1dCM element acquired the capability to initiate maturation. The mechanism is obscure at present, but one possibility is that this ability might have been gained by rescue with the genetic material of *E. coli*, since the genetic material of phage P1 is more homologous with *E. coli* than *S. typhi*.

The present and previous (16) studies demonstrate the alternate association of the CM^r determinant derived from an R factor with different genetic elements, first with phage P1 and then with the F factor. The hybrid elements formed are very stable and behave in several instances as a single unit after the association has occurred. Thus, the genetic interaction between three kinds of episomic elements, the F and R factors, and prophage P1 has been established.

It was reported from this laboratory (9) that the nontransferable R(TC) character, obtained by transduction with ϵ phage and integrated on the host chromosome, acquired conjugal transferability by the formation of F-(TC).

Infection of *Salmonella* strains by F and F' factors, and their hybridization with *E. coli* strains, have been reported (3, 27, 32, 33). The presence of sex factors in *Salmonella* was recognized by the ability of these strains to transfer the factors back to *E. coli*, or by mating with other strains of *Salmonella*. Zinder (33) proposed a staining method for following the transfer of the F agent to *Salmonella*, but it was noted that F⁺ *Salmonella* did not give a positive reaction. Although production of male substances (29) by the hybrid of *Salmonella* with *E. coli* was observed (8, 23), susceptibility of these hybrid strains to f phage was not demonstrated. In this paper,

it was shown that *S. typhi* carrying the F factor is susceptible to f phages.

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