JOURNAL OF VIROLOGY, Jan. 1971, p. 168-175
Copyright © 1971 American Society for Microbiology

Vol. 7, No. 1
Printed in U.S.A.

Isolation and Characterization of a New Generalized Transducing Bacteriophage Different from P1 in *Escherichia coli*

KATSUTOSHI MISE
First Department of Bacteriology, National Institute of Health, Kamiosaki-2-10-35, Shinagawa-ku, Tokyo, Japan

Received for publication 21 September 1970

A new generalized transducing bacteriophage in the *Escherichia coli* system was isolated and characterized. This phage, designated D108, makes clear plaques on *E. coli* K-10, K-12, K-12(P1kc), K-12(D6), B/r, C, and 15 T^-, and *Shigella dysenteriae*. The plaque of phage D108 is larger in size than that of phage P1kc. Electron-microscopic observation revealed that phages D108 and P1kc are morphologically different from each other, suggesting that phage D108 belongs to a phage group different from phage P1. The fact that all of the 10 markers tested were transduced by phage D108 indicates that this phage is a generalized transducing phage in the *E. coli* system. The transduction frequency by phage D108 of chromosomal markers and of a drug resistance factor (R factor) ranged from 2×10^{-6} to 3×10^{-8} and 3×10^{-9} to 6×10^{-10} per phage, respectively. The cotransduction frequency of the *thr* and *leu* markers was 2.8% for phage P1kc and 1.5% for phage D108. The *CM* and *TC* markers (chloramphenicol-resistant and tetracycline-resistant markers, respectively) of the R factor were not cotransduced by phage D108, but the markers were generally cotransduced by phage P1kc. The results suggest that the transducing particle of phage D108 contains a smaller amount of host deoxyribonucleic acid than does phage P1kc.

Since the discovery of phage P22 by Zinder and Lederberg (15), various generalized transducing phages have been isolated by many workers. In the *Escherichia coli* system, three generalized transducing phages, designated P1, 363, and D6, were isolated and used for genetic analysis of *E. coli*. Phages P1 and 363 were isolated from lysogenic strains of *E. coli* (4, 5), whereas phage D6 was isolated from a lysogenic strain of *Salmonella oranienburg* (7). All of the three phages belong to the same phage group, since their biological and serological characters are very similar to each other (4, 7). We intended to find a generalized transducing phage in *E. coli*, the transducing particle of which may contain host deoxyribonucleic acid (DNA) in an amount either smaller or larger than phage P1. Such a phage, when isolated, will be very useful for genetic analysis of *E. coli*, especially for obtaining a more precise genetic map of its chromosome.

In spite of its ability to transduce genetic markers to recipient cells at relatively high frequencies, the P1-group phage is not an ideal phage for transduction experiment for the following reasons. (i) The plaque of the P1-group phage is quite small in size. The phages of this group are, therefore, not very suitable for certain experiments. (ii) Phage lysates containing plaque-forming units (PFU) of more than 3×10^{9}/ml cannot be obtained by liquid incubation. The Lennox method (5) for obtaining a high titer of P1-phage lysate is troublesome and time consuming. (iii) Phage P1 deteriorates considerably when shaken in $CHCl_3$-containing medium.

One of the purposes of this investigation is to isolate a new generalized transducing phage without such defects. To obtain such a phage, we tested various temperate phages for their transducing abilities and isolated one generalized transducing phage which we designated D108. Although the frequency of transduction mediated by phage D108 was lower than that by phage P1, phage D108 had some advantages over phage P1 in genetic analysis. This report describes isolation and characterization of phage D108.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The bacterial strains used are listed in Table 1. The R factor employed was NR1 (8), which is essentially the same as

TABLE 1. *List of bacterial strains*

Bacterial strains	Relevant characters[a]	Reference
Escherichia coli K-12 C600	F^-, λ^-, *lac*⁻, *thr*⁻, *leu*⁻, B_1^-	1
E. coli K-12 C600 (NR1)	C600 with the R factor NR1	Obtained in our laboratory
E. coli K-12 C600 (P1kc)	P1-lysogenic strain of C600	Given by H. Ikeda
E. coli K-12 C600 (D6)	D6-lysogenic strain of C600	7
E. coli K-12 C600 (D108)	D108-lysogenic strain of C600	
E. coli K-12 CS2	F^-, λ^+, lac^+, met^-	11
E. coli K-12 CS2 (NR1)	CS2 with the R factor NR1	6
E. coli K-12 W3623	F^-, λ^-, trp^-	Given by A. Miura
E. coli K-12 W677	F^-, λ^-, lac^-, xyl^-, thr^-, leu^-, B_1^-	2
E. coli K-12 T214	his^-	6
E. coli K-12 T215	ser^- or gly^-	Given by R. Nakaya
E. coli K-12 B/r	Prototrophic	Given by T. Horiuchi
Shigella dysenteriae Sh	F^-	5
Salmonella typhimurium LT2	F^-, lac^-	15

[a] All of the *E. coli* strains except C600 (NR1) and CS2 (NR1) were sensitive to chloramphenicol (CM), streptomycin (SM), and tetracycline (TC).

R factors 222 (14), R4 (9), or R100 (12), independently named by Japanese workers. The bacteriophages used were P22, T4, P1kc (5), and D6 (7). The latter two phages are generalized transducing phages in the *E. coli* system. Phage λ was obtained by ultraviolet irradiation of a λ-lysogenic strain CS2 (λ^+).

Media. Modified L broth, modified L broth-agar, and soft agar were used for growth and titration of phage, respectively. Modified L broth (designated hereafter as L broth) contained 10 g of Daigo peptone, 3 g of yeast extract, and 5 g of NaCl, in 1,000 ml of water. $CaCl_2$ solution was added to L broth before pouring to a final concentration of 2.5×10^{-3} M. The *p*H of the broth was adjusted to 7.2 with 1 M NaOH. Modified L broth-agar (designated hereafter as L broth-agar) contained 6 g of Oxoid agar in 1,000 ml of L broth supplemented with $CaCl_2$ at 2.5×10^{-3} M. Soft agar contained 3.5 g of Oxoid agar in 1,000 ml of L broth supplemented with $CaCl_2$ at 2.5×10^{-3} M. Minimal agar (10), Pennassay Broth (PAB; Difco), and PAB agar were used for transduction experiments. The minimal agar used for the transduction experiments of chromosomal markers contained 10.5 g of K_2HPO_4, 0.4 g of sodium citrate·$2H_2O$, 4.5 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of $(NH_4)_2SO_4$, 0.01 g of 2,3,5-triphenyl tetrazolium chloride, and 10 g of Oxoid agar, in 1,000 ml of water. Glucose, xylose, or lactose was added as a carbon source at a concentration of 0.2, 0.5, or 1.0%, respectively. Vitamins, amino acids, and antibiotics, if necessary, were added at concentrations of 1, 40, and 25 μg/ml, respectively. PAB agar supplemented with chloramphenicol (CM) at 25 μg/ml or tetracycline (TC) at 25 μg/ml was used for transduction experiments with the R factor. EC medium (Eiken) used for growth of *E. coli* in sewerage contained 20 g of polypeptone, 5 g of lactose, 1.5 g of bile salt, 4 g of K_2HPO_4, 1.5 g of KH_2PO_4, and 5 g of NaCl, in 1,000 ml of water.

Chemicals. CM, TC, and streptomycin (SM) were given by S. Yamazaki of our Institute. Mitomycin C was the product of Sankyo Co., Japan. 2,3,5-Triphenyl tetrazolium chloride was the product of Merck & Co., Inc., Rahway, N. J.

Isolation of phage D108. Phage D108 was isolated from a mixed sewerage culture by the following methods. One milliliter of sewerage was pipetted into a culture tube containing 10 ml of EC medium, and the mixture was incubated overnight at 44.5 C without aeration. Only the cells of the *E. coli* group can grow on the medium under these conditions. The cells were then collected by centrifugation, suspended in L broth containing mitomycin C (10 μg/ml) and incubated for 10 min at 37 C with aeration. The mitomycin-treated cells were then centrifuged, washed three times with L broth, and incubated for 2 to 4 hr at 37 C with aeration to induce phages from lysogenic strains. The sample was mixed with a small amount of $CHCl_3$, and the mixture was vigorously shaken to kill bacteria. The mitomycin-induced phage lysate thus obtained was diluted with L broth and mixed with the host bacteria (*E. coli* K-12 C600 or W677) in soft agar; the mixture was plated on L-broth-agar plate. Several types of phage plaques appeared on the plate after overnight incubation at 37 C. These phages were isolated, grown on *E. coli*. K-12 C600 or W677 by liquid incubation, and tested for their transducing ability of the *met* gene. (The recipient strain used was *E. coli* K-12 CS2.) Of approximately 100 phages isolated, only one showed ability to transduce. The phage, designated D108, was investigated further.

Density-gradient centrifugation. A 0.01-ml amount of phage D108 lysate was suspended in 3 ml of CsCl-0.01 M tris(hydroxymethyl)aminomethane solution, and the mixture was centrifuged in the SW50L rotor of a Spinco ultracentrifuge for 17 hr at $100,000 \times g$ and 3 C. Phage P22 was used as a density marker (1.51 g/ml). The sample was fractionated, each fraction was diluted with saline and mixed with soft agar containing the host bacteria, and the mixture was plated on L broth-agar plate. The plaques on the plate were counted after 24 hr of incubation at 37 C.

Electron microscopy. Approximately 300 ml of phage D108 lysate (about 2×10^{11} PFU/ml) obtained by liquid incubation was centrifuged for 20 min at 8,000 rev/min and 2 C to remove bacterial debris. The supernatant was centrifuged in a Spinco ultracentrifuge (model L2, rotor 42) for 65 min at 21,000 rev/min and 5 C. The phages sedimented were suspended in 3 ml of L broth and recentrifuged for 20 min at 8,000 rev/min and 2 C to remove the residual debris. The phage solution thus obtained was mixed with CsCl (2.3 ml of phage solution + 1.75 g of CsCl), and the mixture was centrifuged in the SW39 rotor at 30,000 rev/min for 17 hr at 5 C. After completion of the centrifugation, the visible band of the phage was collected by fractionation. The purified phage sample (3×10^{12} PFU/ml) was diluted with deionized water immediately before electron-microscopic observations and applied to a carbon-coated grid. The grid was stained with phosphotungstic acid (PTA).

Inactivation of phage by an anti-P1 serum. One milliliter of phage lysate (2×10^8 to 6×10^8 PFU/ml) and 0.1 ml of an anti-P1 serum were mixed with 8.9 ml of L broth, and the mixture was incubated at 37 C without aeration. At intervals (5, 10, 15, and 20 min), a 0.1-ml amount of the sample was taken, diluted with L broth, mixed with soft agar containing the host bacteria, and plated on L broth-agar.

Transduction experiments. Transduction experiments were carried out by a slight modification of the procedure of Lennox (5). Recipient cells were grown in L broth to a concentration of 5×10^8 to 8×10^8/ml, collected by centrifugation, and suspended in L broth to a concentration of 5×10^9/ml or more. One to two milliliters of the recipient cells and the same volume of phage lysate (10^9 to 10×10^9 PFU/ml) were pipetted into a culture tube containing 6 to 8 ml of $CaCl_2$-containing L broth to a total volume of 10 ml. After the mixture was incubated for 20 min at 37 C with aeration, the cells in the mixture were collected by centrifugation, suspended in saline, diluted appropriately, and plated on the agar plates suitable to select transductants. The plates were then incubated for 48 to 72 hr at 37 C. A multiplicity of infection (MOI) was usually kept less than 0.5 to prevent the killing effect due to simultaneous infection by an active phage(s).

In each transduction experiment, two control tests were always set up. (i) Phage lysate was plated on L broth-agar or PAB agar for sterility test. (ii) Noninfected culture was tested for spontaneous mutants. Transduction frequency was expressed as the number of transductants divided by the number of phage added.

Transfer of the R factor by conjugation. The methods of the R-factor transfer by mixed cultivation was carried out by the procedure of Rownd et al. (10), except that the mixture containing donor and recipient cells were incubated for 24 hr without aeration.

RESULTS

General characterization of phage D108. Phage D108 made clear plaques on *E. coli* K-12. The plaque of phage D108 was larger in size than that of P1kc, but smaller than that of λ (Fig. 1).

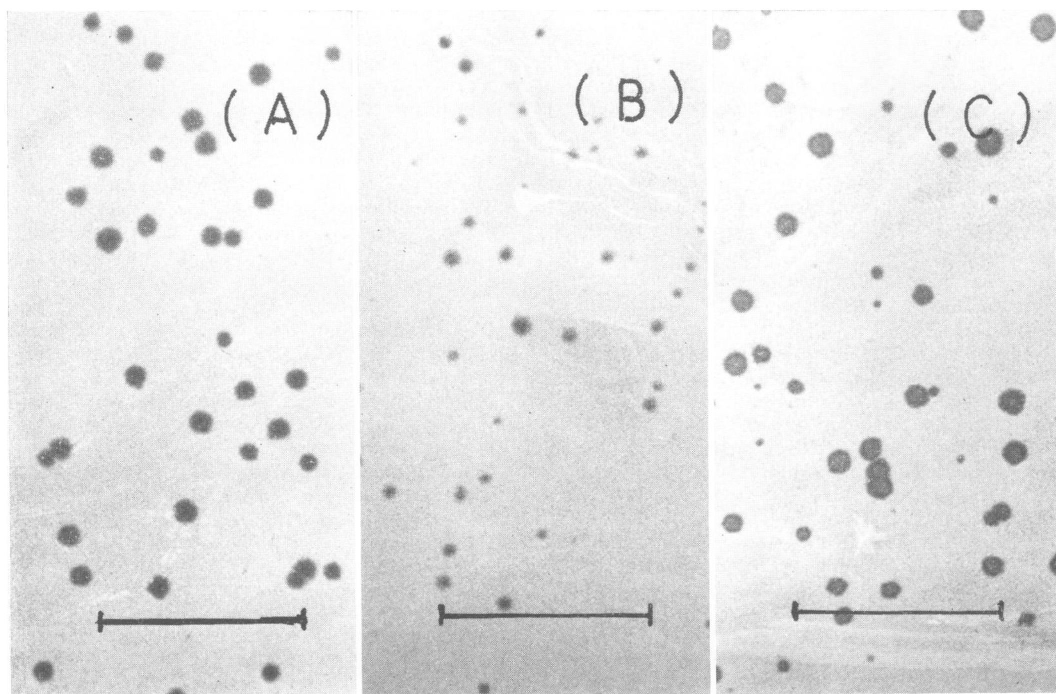


FIG. 1. *Plaques formed on E. coli K-12 C600 by phage D108 (A), P1kc (B), and λ (C). The bars indicate 1 cm.*

D108-lysogenic strains were easily obtained by stabbing a plaque and streaking it on L broth-agar plate. A high titer of phage D108 lysate (5×10^{10} PFU/ml) was easily obtained by incubating the mixture of the phage and *E. coli* K-12 in L broth containing $CaCl_2$ (2.5×10^{-3} M) with aeration. Calcium ion was required for growth of both phage D108 and P1kc. The buoyant densities in CsCl solution of phages D108 and P1kc were almost the same (1.47 g/cm^3). Phage D108 made plaques on *E. coli* K-10, K-12, B/r, C, and 15 T$^-$, and *Shigella dysenteriae* Sh, although at a reduced efficiency on some of the strains. The host ranges of phages D108 and P1kc were different from each other. Phage D108 made plaques on *E. coli* K-12 C600(P1kc) and C600(D6), but not on C600(D108). On the other hand, phage P1kc made plaques on C600(D108), but not on C600(D6) (Table 2). An anti-P1 serum inactivated phage D108, although at a very reduced rate, indicating that there are some significant relationships between P1 and D108 (Fig. 2).

Electron micrograph of phage D108. Figures 3A and B illustrate the shape of phage D108. The diameter of the hexogonal head of D108 is about 6.5 μm and the length of tail is about 13.7 $\times$ 2.2 μm. The tail sheaths of both phages, D108 and P1 are contractile. Phage D108 is smaller than phage P1, with a head diameter of 9 μm and a tail length of 22 $\times$ 2 μm. The ratio of the length of the phage tail to the diameter of the head is about 2.1 for phage D108 and 2.4 for phage P1. The ratio of the length to the width of the tail is about 6 for phage D108 and about 11 for phage P1. The results suggest that phage D108 belongs to a phage group different from phage P1.

TABLE 2. *Plaque-forming ability of phages D108 and P1kc on various bacterial strains*[a]

Bacterial strains[b]	Plaque-forming ability[c]	
	D108	P1kc
C600	1.0	1.0
C600 (D6)	0.54	$<2.5 \times 10^{-9}$
C600 (D108)	$<1.8 \times 10^{-9}$	1.0
C600 (P1kc)	5.5×10^{-3}	$<2.5 \times 10^{-9}$
CS2(λ)	0.85	1.0
T223	1.0	0.9
B/r	8.6×10^{-5}	1.6×10^{-4}
LT2	$<1.8 \times 10^{-9}$	$<2.5 \times 10^{-9}$

[a] The phage lysate used in these experiments was obtained by incubating the mixture of the phage and *E. coli* K-12 C600 with aeration.

[b] The characters of the bacterial strains are described in Table 1.

[c] The plaque-forming ability was expressed as the number of plaque on a test strain per that on *E. coli* K-12 C600.

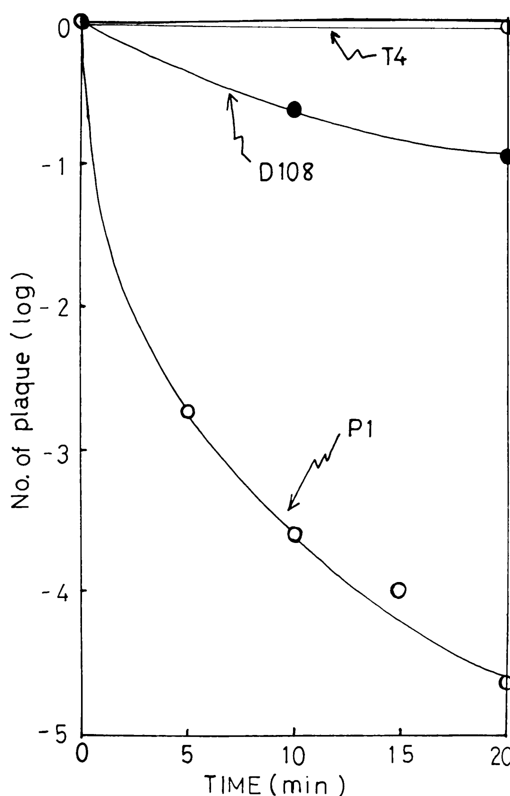
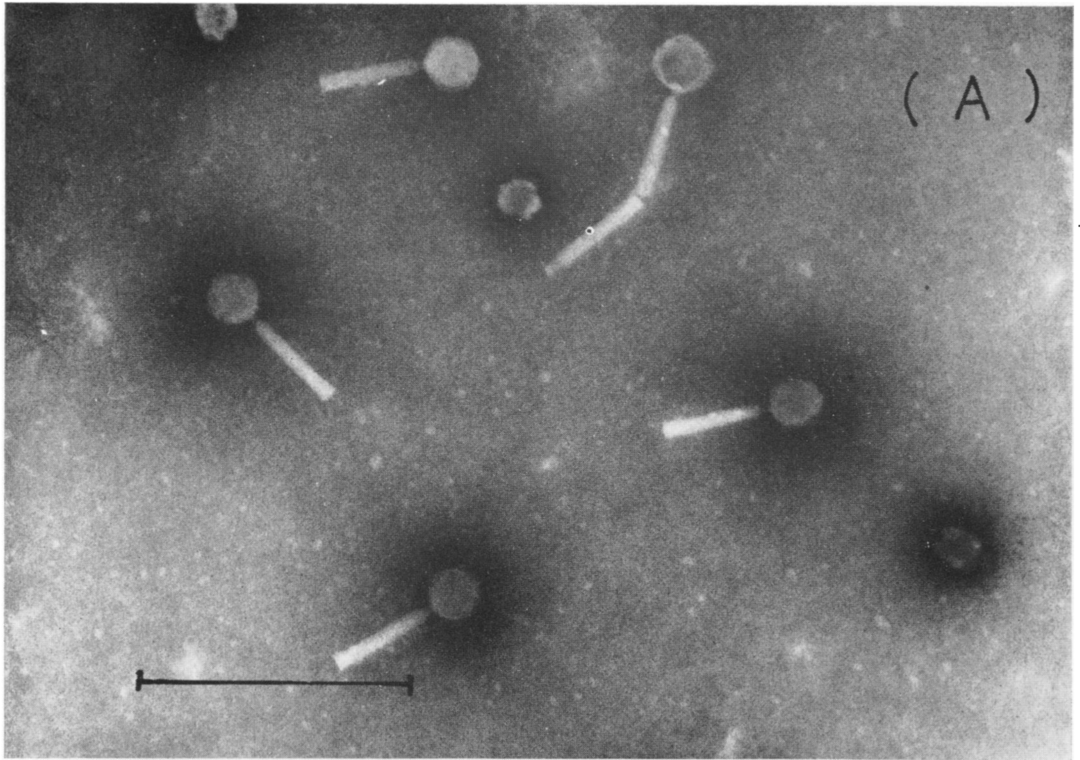


FIG. 2. *Effect of an anti-P1kc serum on phages D108 and P1kc. The L broth containing the phage and the serum (100-fold dilution) was incubated at 37 C for the appointed times without aeration. The host strain used was E. coli K-12 W3623. The plaque number versus time are plotted by setting the plaque number at 0 min as 0. For a control experiment on phage inactivation by a rabbit serum itself, an anti-CM acetyltransferase serum was employed. Phages D108 and P1kc were relatively stable in the 100-fold dilution serum; only 11 and 15% of phages D108 and P1kc, respectively, were inactivated by the serum for 20 min of incubation at 37 C.*

Transduction of various genetic markers by phage D108. All of the various genetic markers, including markers on the R factor, were transduced by phage D108. Representative results are presented in Table 3. The frequency of transduction of the chromosomal markers and the R factor ranged from 2×10^{-6} to 3×10^{-8} and 3×10^{-9} to 6×10^{-10} per phage, respectively. The higher the MOI, the lower the frequency of transduction. Although phage D108 is a temperate phage, the lytic potential of the phage on *E. coli* K-12 was very high. More than 99% of *E.*



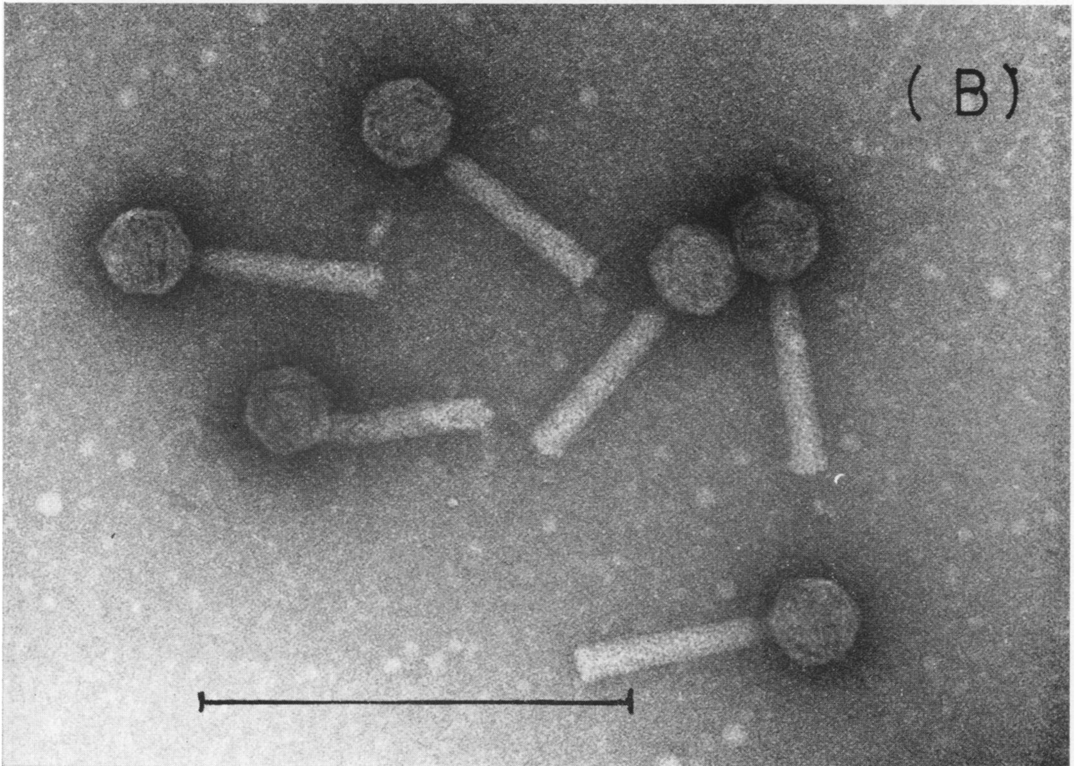


FIG. 3 A and B. *Electron micrographs of phage D108. PTA-negative staining. The bars indicate 30 μm. A D108 virion without a head but with a tail sheath can be seen. An unknown material which may be the degraded head of phage D108 or the debris of the host bacteria can also be seen.*

TABLE 3. *Transduction of various genetic markers with phage D108*[a]

Donor	Recipient	Selected marker[b]	Transduction frequency	MOI[c]
C600 (NR1)	CS2	*met*	1.2×10^{-7}	5
C600 (NR1)	CS2	*met*	1.9×10^{-6}	0.1
C600 NR1)	W3623	*trp*	7.1×10^{-8}	0.2
C600 (NR1)	T214	*his*	4.0×10^{-8}	0.1
C600 (NR1)	T215	*ser* or *gly*	3.6×10^{-8}	0.3
W3623	W677	*thr*	2.8×10^{-7}	0.5
W3623	W677	*leu*	4.0×10^{-7}	0.5
W3623	W677	*lac*	3.2×10^{-7}	0.5
W3623	W677	*xyl*	1.4×10^{-7}	0.5
C600 (NR1)	CS2	*CM*	3.0×10^{-9}	0.1
C600 (NR1)	CS2	*TC*	2.7×10^{-9}	0.1
CS2 (NR1)	CS2	*CM*	6.0×10^{-10}	1.5
CS2 (NR1)	CS2	*TC*	1.2×10^{-9}	1.5

[a] The detailed method for transduction experiments is described in the text. Phage D108 was obtained by incubating the mixture of the phage and the donor strain with aeration. In each experiment, the following two controls were always run: (i) phage lysate was plated on L broth agar or PAB agar for sterility test; (ii) noninfected culture was assayed for spontaneous mutants. In the transduction experiments with the R factor, we tested the transduction frequency of the selected marker only.

[b] *CM*, resistant to chloramphenicol; *TC*, resistant to tetracycline.

[c] Multiplicity of infection.

coli K-12 C600 was killed by phage D108 during 1 hr of incubation at an MOI of 2.5 at 37 C. The reduced transduction frequency at a high MOI is because the bacterial cells transduced are killed by simultaneous infection of an active particle(s) of phage D108. Accordingly, a large number of recipient cells are required for transduction experiments with phage D108. The transduction frequencies of various chromosomal markers mediated by phage D108 were variable among the markers, and were 1 to 100 times lower than the frequencies mediated by phage P1kc as compared with the data published elsewhere (3, 5). The transduction frequency of the R factor NR1 by phage D108 was about 100,000 times lower than that by phage P1. (The transduction frequencies of the *CM* and *TC* markers by phage P1 were 3.0×10^{-4} and 2.7×10^{-4} per phage, respectively.)

The number of the D108-mediated transductants selected on the TC plate or the CM plate were counted after 48 or 72 hr of incubation, since about 70% of the transductants did not form visible colonies on the plate within 24 hr of incubation. On the other hand, the P1-mediated transductants receiving the resitance gene(s) of the R factor were counted during a 24-hr incubation period since all of the transductants formed colonies on the plate within 24 hr. The reason most of the D108-mediated transductants appeared on the plate later than 24 hr remains unknown.

Amount of host genome carried by phage D 108. To examine the amount of the host genome carried by the transducing particle of phage D108, the cotransduction frequencies of the markers *thr* and *leu* by phage D108 and phage P1kc were compared. The frequencies of cotransduction of the markers *thr* and *leu* were 2.8% for phage P1kc and 1.5% for phage D108 (Table 4). More pronounced differences between the P1- and D108-transduction systems were found in transduction experiments with the R factor. The markers *CM* and *TC* of the R factor were generally cotransduced by phage P1kc, whereas the markers were never cotransduced by phage D108 (Table 5). Moreover, almost all of the P1kc-mediated transductants selected on TC plates possessed the conjugal transferability of the *TC* marker; however, none of the D108-mediated transductants selected on the plates possessed the

TABLE 4. *Cotransduction of the thr and leu markers by phages D108 and P1kc*[a]

Phage	Selected marker	Unselected marker tested	No. of colonies transduced for selected marker	No. of colonies cotransduced	Frequency of cotransduction (%)
D108	*leu*	*thr*	1883	26	1.5
P1kc	*leu*	*thr*	1656	46	2.8

[a] Donor strain and recipient one were *E. coli* K-12 W3623 and *E. coli* K-12 W677, respectively.

TABLE 5. *Cotransduction of the CM and TC markers of the R factor by phages D108 and P1kc*[a]

Phage	Selected marker	Unselected marker tested	No. of colonies transduced for selected marker	No. of colonies cotransduced	Frequency of cotransduction (%)
D108	*TC*	*CM*	150	0	0
P1kc	*TC*	*CM*	160	159	99.4

[a] Donor and recipient were *E. coli* K-12 C600 (NR1) and *E. coli* K-12 CS2, respectively.

ability. It was reported that the transducing particles of phage P1kc lack phage genome and carry only fragments of the host genome corresponding to a molecular weight of 6×10^7 daltons (3). The cotransduction experiments on the *leu* and *thr* markers and the R factor suggest that the host genome of the transducing particle of phage D108 is smaller in size than that of phage P1kc; that is, the molecular weight of the host genome carried by phage D108 is below 6×10^7 daltons

In the experiments with R factor 222 (the same R factor as NR1; *see* Materials and Methods), Watanabe and Fukasawa reported that all of the P22-mediated transductants of LT-2 selected on the CM plate were also resistant to SM but sensitive to TC (13, 14). From the results, they postulated that the *SM* marker (streptomycin-resistant marker) of the R factor is located near the *CM* marker. This postulation was supported by our finding that the *CM* marker of the R factor was always cotransduced with the *SM* marker by phage D108, but that the *TC* marker was not cotransduced with the *SM* marker by the phage thus far tested.

DISCUSSION

A new generalized transducing phage D108, described in this report, has some favorable characters for transduction experiments. (i) The plaque of phage D108 is larger in size than that of phage P1. (ii) A relatively high titer of phage lysate (5×10^{10} PFU/ml) is easily obtained by liquid incubation. The titer of phage P1 lysate obtained by liquid incubation is always below 3×10^9 PFU/ml. (iii) Cotransduction experiments on phage D108 showed that the amount of the host genome carried by phage D108 is smaller than that carried by phage P1.

As compared with phage P1, the unfavorable characteristics of phage D108 as a generalized transducing phage in *E. coli* are as follows. (i) The frequency of transduction is relatively low. (ii) The virulence of the phage to *E. coli* K-12 is so high that a large number of recipient cells are required for transduction experiment. A mutant phage of D108 with low virulence and high transducing ability, if isolated, will be very useful for transduction experiments with *E. coli*. In spite of the fact that phage D108 transduces genetic materials at a relatively low frequency, it will help investigators make more detailed genetic analyses of *E. coli*, since the amount of the host genome carried by phage P1 and D108 is not the same, and since generalized transducing phages which belong to a phage group different from P1 have not been isolated. Unlike P1, phage D108 does not transduce the whole molecule of the R factor. Thus, various incomplete types of the R factor, which are useful for genetic analysis of the R factor, can be obtained from the transductants mediated by this phage. Although neither phage P22 nor phage D108 transduces the whole molecule of the R factor, the P22-LT2 system is not very suitable for genetic analysis of the R factor, since the level of the antibiotic resistance of LT2 (R^-) (*S. typhimurium* LT2 without the R factor) is considerably high as compared with that of K-12 (R^-). [For example, the level of SM resistance is about 100 μg/ml for LT2 (R^-) and 5 μg/ml for K-12 (R^-).] We believe phage D108 will provide a useful tool to the investigators who have been working on genetic aspects of the R factor.

The transduction frequency by phage D108 of the resistance markers of R factor NR1 is extremely low, with a range of 3×10^{-9} to 6×10^{-10} per phage. This may well be explained, if we assume that the transducing particle of phage D108 cannot contain the whole molecule of the R-factor DNA and that the resistance genes of the R factor is not closely linked to the gene(s) necessary for replication of the R factor. Therefore, some of the resistance genes cannot be cotransduced with the replication gene(s) of the R factor by phage D108. The R factor with the resistance genes but without the replication gene(s), if transduced by phage D108, will be eliminated from a recipient cell by growth of the cell. Further investigations are necessary for verification of our assumption.

ACKNOWLEDGMENTS

We acknowledge the advice and help of A. Ohsaka and C. Yamada throughout this work.

We are also grateful to K. Suzuki for taking electron micrographs of phage D108.

ADDENDUM IN PROOF

After submission of our manuscript, we became aware of the existence of a report by H. Drexler (Proc. Nat. Acad. Sci. U.S.A. 66:1083, 1970) stating that amber mutants of virulent phage T4 are able to transduce a wide variety of genetic markers from permissive to nonpermissive K strains of *E. coli*.

LITERATURE CITED

1. Appleyard, R. K. 1954. Segregation of new lysogenic types during growth of doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
2. Clowes, R. C., and D. Rowley. 1954. Some observations on linkage effects in genetic recombination in *E. coli* K12. J. Gen. Microbiol. **11**:250–260.
3. Ikeda, H., and J. Tomizawa. 1965. Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. J. Mol. Biol. **14**:85–109.
4. Jacob, F. 1955. Transduction of lysogeny in *Escherichia coli*. Virology **1**:207–220.
5. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
6. Mise, K., and Y. Suzuki. 1968. Temperature-sensitive chlor-

amphenicol acetyltransferase from *Escherichia coli* carrying mutant R factor. J. Bacteriol. 95:2124–2130.
7. Mise, K., and K. Suzuki. 1970. New generalized transducing bacteriophage in *Escherichia coli*. J. Virol. 6:253–255.
8. Nakaya, R., A. Nakamura, and Y. Murata. 1960. Resistance transfer agents in *Shigella*. Biochem. Biophys. Res. Commun. 3:654–659.
9. Okamoto, S., and Y. Suzuki. 1965. Chloramphenicol-, dihydrostreptomycin-, and kanamycin-inactivating enzymes from multiple drug-resistant *Escherichia coli* carrying episome "R." Nature (London) 208:1301–1303.
10. Rownd, R., R. Nakaya, and A. Nakamura. 1966. Molecular nature of the drug-resistance factors of the *Enterobacteriaceae*. J. Mol. Biol. 17:376–393.
11. Skaar, P. D., and A. Garen. 1956. The orientation and extent of gene transfer in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. 42:619–624.
12. Sugino, Y., and Y. Hirota. 1962. Conjugal fertility associated with resistance factor R in *Escherichia coli*. J. Bacteriol. 84: 902–910.
13. Watanabe, T., and T. Fukasawa. 1960. "Resistance transfer factor" An episome in *Enterobacteriaceae*. Biochem. Biophys. Res. Commun. 3:660–665.
14. Watanabe, T., and T. Fukasawa. 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. III. Transduction of resistance factors. J. Bacteriol. 82:202–209.
15. Zinder, N. D., and J. Lederberg. 1952. Genetic exchange in Salmonella. J. Bacteriol. 64:679–699.