Inactivation of Nocardiphages ϕ C and ϕ EC by Extracts of Bacteriophage-Attachable Cells

GEORGE H. BROWNELL AND JENNIFER K. CROCKETT

Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30902

Received for publication 11 August 1971

Cultures of several species of *Nocardia*, including *N. erythropolis* Mat-Ce and Mat-cE mating strains, were extracted with solvents in an attempt to isolate an inactivating complex for nocardiphages ϕ C and ϕ EC. Ethanol was the only solvent found effective in solubilizing an inhibitory substance. Inactivating extracts were obtained from the cells of all species to which the phage were able to attach. After extraction of whole cells or cell wall preparations, the phage could not effectively attach to them. Both phages ϕ C and ϕ EC were inactivated by the same complex. However, phage ϕ EC inactivation was 10-fold greater than ϕ C inactivation. The velocity of inactivation was about 4.1×10^2 plaque-forming units per microgram per minute for ϕ C and 1.1×10^8 plaque-forming units per microgram per minute for phage ϕ EC. The cell extracts required divalent cations for phage inactivation. The inhibitory capacity of the cell extracts was reduced or lost by the activity of proteolytic enzymes, Tween 80, 2-mercaptoethanol, thymol, and sodium lauryl sulfate. Boiling the extract for 10 min did not alter its activity. The inactivating substance was postulated to be a lipoprotein of considerable complexity, unique in the ease with which it is solubilized from host cells by ethanol.

The extraction of phage-inhibiting complexes or receptor substances has been demonstrated in a number of genera and, in some instances, the chemical nature of the inactivator has been ascertained (6, 10). In the mycobacteria, Tokunaga et al. (8, 9) reported that ethanol-ether (1:1) extracts of *Mycobacterium smegmatis* inactivated several mycobacteriophages. On the basis of the effects of various physical and chemical agents on the extract, they postulated that either a lipoprotein or a lipopolysaccharide complex was an important component of the receptor substance.

This report deals with the inactivation of two nocardiphages isolated from the soil (5). One phage, ϕ C, infects the Mat-Ce mating strain of *Nocardia erythropolis*, whereas the second phage, ϕ EC, infects both the Mat-Ce and Mat-cE mating strains. Recently, a mutant of *N. restrictus*, AY B-311, was found to form plaques with both phages ϕ C and ϕ EC with soft-agar assaying techniques. The sensitivity to phage ϕ C has been exploited as a genetic marker (4), and, while it is not mating type-specific, it is the most convenient means for rapid differentiation of the wild-type mating strains in the *N. erythropolis* system. The phage ϕ EC has been shown to be a temperate phage. Clones lysogenic for phage ϕ EC have been isolated from both *N. erythropolis*

mating type strains (*unpublished data*). The present report establishes the isolation, inactivation, and preliminary characterization of a phage inhibitor complex from a group of nocardiae related either genetically or by their sensitivity to the phages ϕ C and ϕ EC.

MATERIALS AND METHODS

Cultivation of organisms and propagation of phage. The organisms were cultured in either peptone-yeast extract (PY) broth (Difco) or in minimal medium (MM) broth (1) for 24 to 36 hr at 30 C in a shaker incubator. Stock preparations of both nocardiphages ϕ C and ϕ EC were propagated in cultures of *N. erythropolis* Mat-Ce (5). The phage were tested for their ability to attach to and to propagate in the organisms employed in this investigation. Phage stock suspensions were added to PY broth cultures of the organisms after 24 to 36 hr of incubation. After 6 to 12 hr of incubation, the cultures were centrifuged, and the supernatant fluid from each was filtered and assayed for phage. All phage assays were done by the standard soft-agar overlay technique (2). To determine attachment, cultures were centrifuged and cells were suspended in fresh PY to an absorbance of 0.5 at 620 nm. A phage stock suspension containing 10^8 plaque-forming units (PFU) per milliliter was added to the cell suspensions. After 10 min, the cultures were filtered, and the filtrate was assayed for phage. The per cent attachment was obtained by comparing the initial phage concentration with that of the free

phage after the adsorption period. Attachment was also assayed by determining the percentage of phage resistant to specific antiserum. Phage able to form plaques after the addition of antiserum were considered to be attached to the cells. Ethanol-extracted cultures were washed in PY broth and standardized as above. Attachment to these extracted cells was determined by the percentage of free phage.

Sensitivity of phage to solvents. Various solvents were tested for their effects on the phage. The solvents to be employed in cell extractions were added to a stock suspension of phage containing 10^8 PFU/ml to a final concentration of 10% (v/v) in PY broth. The mixtures were incubated for 30 min at 30 C. Dilutions were made in PY broth, and the per cent phage sensitivity was obtained by assaying with *N. erythropolis* Mat-Ce host.

Cell extraction and phage inactivation tests. In some instances, whole cells were extracted after three saline washes or after lyophilization, and, in other experiments, cell walls were isolated before extraction. Cell walls were obtained by first disrupting cells in a Braun homogenizer (Bronwill Scientific Inc., Rochester, N.Y.). Approximately 10 g of wet cells was placed together with 20 g of glass beads (0.45 mm) and shaken for 10 to 15 min. A CO₂ cooling device was employed to prevent excessive heating. The mixture was then centrifuged at $20,000 \times g$ for 10 min. The cell debris layer was removed, washed with saline (0.85%), and centrifuged. The washing procedure was repeated until a Gram stain revealed a homogeneous cell wall suspension. For extraction, 1 g of lyophilized or 10 g of wet whole cells was suspended in 30 ml of a given solvent and shaken for 15 to 30 min at 30 C. One gram of wet cell walls was extracted in a similar manner. The mixtures were centrifuged, and the supernatant fluid was evaporated under vacuum in a rotary evaporator. Residues that had been extracted with solvents which demonstrated adverse effects on the phage were redissolved in 10 ml of ethanol. Others were dissolved in 10 ml of the solvent used for extraction.

The inactivating capacity of the cell extracts was initially assayed by adding extract to a final concentration of 10% (v/v) to a stock preparation of phage containing 10^8 PFU/ml in PY broth. At given time intervals, samples were removed, diluted in PY broth, and tested for phage inhibition. In later experiments, it was observed that the inactivating complex could be precipitated by Ca(NO₃)₂. Therefore, the inactivating substance was concentrated and partially purified by adding 5 ml of 0.01 M Ca(NO₃)₂ to the extracted residues which had been dissolved in 10 ml of ethanol. The extract-Ca(NO₃)₂ mixture was incubated for 10 min, centrifuged, and redissolved in 5 ml of ethanol. The yield from this procedure was about 150 µg/ml (dry weight). In another procedure, 15 ml of ether and 15 ml of water were added to the extract. The ether layer was collected, dried over sodium sulfate, evaporated with nitrogen, and dissolved in 5 ml of ethanol. This procedure produced about 200 µg/ml (dry weight). To determine the kinetics of inactivation, extract was added to a final concentration of 2 µg/ml and 20 µg/ml, respectively,

to phage stock preparations, and samples were removed and assayed for phage at various time intervals. To determine if the same component of the cell extract was responsible for the inhibition of both phages ϕ C and ϕ EC, the extracts were preadsorbed with phage. Portions of a high-titer suspension of one phage were added successively to phage-extract mixtures and incubated for 30 min. When no further reduction in phage titers was observed, the extract was considered to be saturated and was then tested for its ability to inhibit the other phage.

Effects of chemical and physical agents. The effects of cations, proteolytic enzymes, detergents, temperature, and several other chemical agents on the inactivation of phage by cell extracts were determined to provide information on the mode of action, the chemical nature, and the complexity of the inhibitor. To assay the effects of cations, a peptone-glucose (PG) broth (1 g of peptone, 3 g of glucose/liter) was used as a diluent. Cations in 0.001 M concentration and cell extract in 10% (v/v) concentration were added to the diluent. A 0.1-ml sample of a stock suspension of phage was added to 0.9 ml of the mixture; the mixture was then incubated and assayed as previously described. The effects of Pronase (Calbiochem, Los Angeles, Calif.) and trypsin (Difco) were assayed in 0.01 M THAM (2-amino-2-hydroxymethyl-1,3-propanediol) buffer (pH 8.0) plus 0.001 M Ca(NO₃)₂ at a concentration of 100 µg/ml. Extract-enzyme mixtures and phage-enzyme control mixtures were incubated for 60 min at 37 C. Phage preparations of about 10^7 PFU/ml in PY broth were added to the extract-enzyme mixtures and assayed as previously described. The effects of the other chemical agents were assayed in PG broth plus 0.001 M Ca(NO₃)₂. The concentrations of each agent tested are given in Table 5.

RESULTS

The ability of the nocardiphages ϕ C and ϕ EC to attach to and to propagate in the organisms employed in this investigation is shown in Table 1. Both phages were found to attach to all organisms effectively, with the exception of *N. corallina* ATCC 4273. The attachment of phage ϕ EC was about 10% more effective than that of ϕ C. Phage ϕ C failed to propagate in the *N. erythropolis* Mat-cE mating strain, in *N. restrictus* ATCC 14887, and in *N. corallina*. The ϕ EC phage propagated in both *N. erythropolis* mating strains but not in *N. restrictus* ATCC 14887 nor in *N. corallina*. Both phages showed a considerable variation in the PFU/ml produced by the various hosts when infected at the time the cells were harvested for extraction.

The effects of solvents and cell extracts obtained with the solvents on the nocardiphage are shown in Table 2. After 30 min of incubation with petroleum ether, diethyl ether, and ethanol, there was little or no loss of phage viability. Butanol, chloroform, acetone, 2-propanol, and methanol reduced phage titers. Chloroform-

TABLE 1. Attachment and propagation of nocardio-phages ϕC and ϕEC

Organism	ϕC		ϕEC	
	Per cent attachment	Phage yield (PFU ^b /ml)	Per cent attachment	Phage yield (PFU/ml)
<i>Nocardia erythropolis</i> Mat-Ce ^a	87.3	3.2×10^7	91.5	1.0×10^6
<i>N. erythropolis</i> Mat-cE ATCC 4277	81.6	0	96.2	1.2×10^6
<i>N. restrictus</i> ATCC 14887	80.3	0	89.0	0
<i>N. restrictus</i> B-311	72.0	3.8×10^3	86.9	6.7×10^4
<i>N. globerula</i> ATCC 9356	88.1	6.7×10^6	95.6	4.3×10^6
<i>N. calcarrea</i> ATCC 19369	74.3	4.3×10^3	92.2	3.1×10^4
<i>N. corallina</i> ATCC 4273	3.9	0	6.1	0

^a Previously designated *N. canicruria* 3 (3).^b Plaque-forming units.

methanol (2:1) was the most effective solvent causing phage inactivation. Extracts of *N. erythropolis* Mat-Ce were tested for their capacity to inactivate the phage (Table 2). Of the various solvents employed, only ethanol was effective in solubilizing an active phage inhibitor from this organism. Both phages ϕC and ϕEC were equally inactivated (99.9%) by the ethanol extract.

Ethanol was also used to extract the other organisms and the cell wall preparations of the two mating strains of *N. erythropolis* (Table 3). Extracts of whole cells and isolated cell walls of both the *N. erythropolis* Mat-Ce and Mat-cE mating strains inactivated phages ϕC and ϕEC . Whole cell extracts of *N. restrictus* wild type and mutant B-311, *N. globerula*, and *N. calcarrea* also inhibited both phages. Only the extracts from *N. corallina* failed to inactivate the phage. The attachment studies showed that the phage could attach to the isolated walls (Table 3). After extraction of either whole cells or cell walls, the ability of the phage to attach to them was greatly reduced. For example, with whole cells of the Mat-Ce strain of *N. erythropolis*, attachment of phage ϕC dropped from 87% before extraction to 23% after extraction and that of phage ϕEC dropped from 91 to 21%.

The rates at which the two nocardio-phages were being inactivated were compared by using extracts from *N. erythropolis* Mat-Ce (Fig. 1). The per cent survival was plotted against the length of incubation for two concentrations of extract with phage ϕC and with phage ϕEC . At both 2 μ g/ml and 20 μ g/ml, the ϕEC phage showed a greater per cent inactivation than did phage ϕC . Both phages required 20 to 30 min for maximum inactivation. The calculated velocity of inactivation is about 4.1×10^2 PFU per μ g per

min for ϕC and 1.1×10^3 PFU per μ g per min for phage ϕEC .

To determine whether phages ϕC and ϕEC were being inactivated by the same complex, the extract was adsorbed with one phage and then tested for its capacity to inactivate the other phage (Table 4). An extract obtained from *N. erythropolis* Mat-Ce initially inhibited phage ϕC by 99.6%. A second dose of ϕC was in-

TABLE 2. Effects of solvents and cell extracts of *Nocardia erythropolis* Mat-Ce on phages ϕC and ϕEC

Solvent	Per cent inactivation of ϕC^a		Per cent inactivation of ϕEC^a	
	Solvent ^b	Cell extract ^c	Solvent ^b	Cell extract ^c
Petroleum ether	0.0	8.4	2.1	7.3
Hexane	6.3	9.3	8.2	10.6
Butanol	16.0	15.1	15.9	11.6
Chloroform ^d	89.8	18.5	97.4	22.3
Diethyl ether	3.0	7.7	1.2	8.4
Acetone	26.3		41.2	
2-Propanol	12.0		56.3	
Ethanol	3.6	99.9	2.4	99.9
Methanol ^d	18.5	24.1	39.6	17.6
Chloroform-methanol ^d (2:1)	99.5	35.0	99.9	37.5

^a Per cent inactivation was determined after 30 min of incubation at 30 C.^b Solvents were added to a concentration of 10% (v/v) to a stock suspension containing 10^8 plaque-forming units per milliliter in peptone-yeast extract broth.^c Cells were extracted with the solvents listed.^d After cell extraction, these solvents were reduced to dryness over nitrogen, and the residues were redissolved in 10 ml of ethanol.

TABLE 3. Cell attachment and extract inactivation of nocardiphages ϕ C and ϕ EC

Organism	ϕ C		ϕ EC	
	Per cent attachment	Per cent inactivation ^a	Per cent attachment	Per cent inactivation ^a
<i>Nocardia erythropolis</i> Mat-cE				
Whole cells	81.6	99.9	96.1	99.9
Cell walls	63.2	98.7	78.8	99.4
Extracted cells	12.4		26.6	
Extracted cell walls	7.4		13.3	
<i>N. erythropolis</i> Mat-Ce				
Whole cells	87.3	99.9	91.5	99.9
Cell walls	76.4	97.0	86.9	99.8
Extracted cells	23.1		21.2	
Extracted cell walls	19.0		14.0	
<i>N. restrictus</i> ATCC 14887				
Whole cells	76.0	90.7	84.6	99.3
Extracted cells	21.0		16.0	
<i>N. restrictus</i> B-311				
Whole cells	72.0	85.0	86.9	97.8
Extracted cells	22.7		24.6	
<i>N. globerula</i> ATCC 9356				
Whole cells	88.1	99.9	95.6	99.9
Extracted cells	23.6		29.7	
<i>N. calcarrea</i> ATCC 19369				
Whole cells	74.3	81.5	92.2	96.1
Extracted cells	17.1		18.3	
<i>N. corallina</i> ATCC 4273				
Whole cells	3.9	3.6	6.1	2.6

^a Per cent inactivation of phage by cell extracts.

hibited by only 21.3%. When phage ϕ C was added to the ϕ EC-adsorbed extract, 66.7% of this phage was inactivated. Preparations of phage ϕ EC were initially inhibited by 99.9%, whereas the inhibition of a second dose was reduced to 63.7%, a figure comparable to the 66.7% inactivation obtained from the ϕ C-adsorbed extract. When ϕ C was added to the ϕ EC-adsorbed extract, only 16.7% of the phage was inactivated, a value similar to that obtained from its own preadsorbed extract.

The effects of cations on the inactivation of the phage by cell extracts are shown in Table 5. Inactivation of phage in the PG broth was less than that observed with PY broth. The addition of monovalent Na and K ions inhibited the inactivation of both phages by the extract. The divalent Ca and Mg ions allowed maximum inactivation by the extract (Table 5).

When the extracts were incubated with pro-

teolytic enzymes, the capacity of the extract to inactivate the phage was altered (Table 5). Pronase and trypsin both reduced the percentage of phage inactivated to 15 to 30%. The detergents which were tested, Tween 80 and sodium lauryl sulfate, also affected the activity of the extracts. A 0.06% solution of Tween 80 prevented extract inactivation of both nocardiphages. Sodium lauryl sulfate reduced inactivation to 31 to 58%.

FIG. 1. Inactivation of nocardiphages ϕ C and ϕ EC by cell extracts of *Nocardia erythropolis* Mat-Ce. Phage suspensions of 10^7 plaque-forming units per milliliter in peptone-yeast extract broth were incubated with 2 μ g/ml and 20 μ g/ml of cell extract at 30 C. Samples were removed and assayed at the indicated time intervals.

TABLE 4. Effects of preadsorbed extracts on nocardiphages ϕ C and ϕ EC

Treatment	Per cent inactivation
ϕ C + extract	99.6
ϕ C + ϕ C-adsorbed extract	21.3
ϕ EC + ϕ C-adsorbed extract	66.7
ϕ EC + extract	99.9
ϕ EC + ϕ EC-adsorbed extract	63.5
ϕ C + ϕ EC-adsorbed extract	16.7

TABLE 5. Effects of chemical and physical agents on inactivation of phage ϕ C and ϕ EC by cell extracts

Treatment ^a	Phage control ^b		Per cent ^c inactivation	
	ϕ C	ϕ EC	ϕ C	ϕ EC
PG broth + 0.001 M $\text{Ca}(\text{NO}_3)_2^d$	2.0×10^{7d}	3.0×10^{6d}	99.7	99.9
Tween 80 (0.06%)	1.0×10^7	1.0×10^6	4.2	8.7
Sodium lauryl sulfate (0.01%)	1.5×10^7	1.8×10^6	31.3	58.6
EDTA ^d (0.001 M)	5.0×10^6	5.0×10^5	2.9	12.1
2-Mercaptoethanol (0.1 M)	1.0×10^7	7.9×10^5	7.5	3.3
Thymol (0.001 M)	8.6×10^6	4.9×10^5	33.0	25.6
Urea (10.0 M)	2.8×10^7	5.1×10^6	99.8	99.9
100 C (10 min)			97.6	99.9
PG broth	1.0×10^{7d}	2.0×10^{6d}	60.3	83.2
NaCl (0.001 M)	7.2×10^6	8.4×10^5	13.1	26.7
CaCl_2 (0.001 M)	1.8×10^7	3.0×10^6	96.7	99.9
MgCl_2 (0.001 M)	1.6×10^7	2.9×10^6	95.0	99.7
KCl (0.001 M)	1.3×10^6	1.0×10^5	17.0	31.2
THAM (0.01 M)	3.0×10^{7d}	5.0×10^{6d}	99.9	99.9
Pronase (100 $\mu\text{g}/\text{ml}$)	9.0×10^6	1.0×10^6	30.1	25.6
Trypsin (100 $\mu\text{g}/\text{ml}$)	1.5×10^7	2.3×10^6	15.9	17.2

^a Abbreviations: PG, peptone-glucose; EDTA, ethylenediaminetetraacetic acid; THAM, 2-amino-2-hydroxymethyl-1,3-propanediol.

^b Effects of the diluents on the phage were determined by adding 0.1 ml of phage stock preparation [10^8 plaque-forming units (PFU) per milliliter, phage ϕ C; 5.0×10^7 PFU/ml, phage ϕ EC] to 0.9 ml of the indicated mixtures. The phage were then incubated for 60 min at 37 C and titered with PG soft-agar overlays.

^c The per cent of inactivation of phage by cell extracts which had been incubated for 60 min at 37 C in the indicated mixtures prior to addition of phage. After addition of phage stocks, the phage-extract mixtures were incubated for another 60 min at 37 C. The per cent inactivation is based on the PFU/ml obtained from the phage-extract mixture treated phage control.

^d Untreated phage controls.

and ethylenediaminetetraacetic acid substantially decreased or eliminated the inactivating ability of the extract. Treating the extracts with urea or boiling the extracts for 10 min failed to alter their inhibitory capacity.

DISCUSSION

Prior to this report, no information as to the ability to extract a phage-inactivating substance from the nocardiae is available. The inactivating complex, presumably a phage receptor, is unique to the nocardiae in the ease with which it is solubilized by ethanol. Extraction of phage receptors from the *Mycobacteria* is accomplished by incubating host cells for 12 hr with such solvents as ethanol-ether (1:1), chloroform-methanol (2:1), chloroform, and 0.5% potassium hydroxide-ethanol (8). These solvent systems and others employed in the present investigation probably solubilize the inactivating complex from the nocardial cell walls. However, only ethanol was effective in solubilizing the receptor so that it could bind to the phage under the conditions of our assay. Once extracted, the inactivating substance could be dissolved in ether, washed with water, and redissolved in

ethanol with only a slight reduction in its inhibitory capacity. When the inhibitory complex was assayed in ether, it failed to inactivate the phage.

Although phage ϕ C fails to propagate in the Mat-cE mating strain of *N. erythropolis*, it does effectively attach to these cells. The same is true for both phages ϕ C and ϕ EC and *N. restrictus* ATCC 14887. The attachment to and propagation of phage in the organisms was determined in 24- to 36-hr cultures at the time cells were harvested for extraction. Cultures at this age are suboptimal for phage yields (5) which accounts for the low titers obtained in the propagation studies (Table 1). Cultures of *N. erythropolis* inoculated with phage after 6 hr of incubation produce titers in the range of 5.0×10^9 PFU/ml (5).

Petroleum ether, hexane, diethyl ether, and ethanol were found to cause no significant loss of phage viability in 10% concentrations for 30 min. Methanol, 2-propanol, and acetone caused 12 to 26% inhibition of phage ϕ C. The same solvents inhibited the ϕ EC phage to the extent of 39 to 56%. Chloroform reduced phage titers by 89 to 97%. Similar solvent sensitivities of myco-

bacteriophages have been interpreted to mean that the phages contain lipoprotein complexes necessary for viability (7). Why these nocardio-phages should be inactivated by chloroform and methanol and unaffected by ether and ethanol is not known.

The inactivating substance was obtained from all cells to which the phage could attach. Isolated cell walls also provided a source of phage-inactivating material. After ethanol extraction, the ability of the phage to attach to either walls or whole cells was greatly reduced. Phages ϕ C and ϕ EC seemed to be inactivated by the same complex. However, the same concentration of inhibitor was able to inactivate about 10-fold more ϕ EC than ϕ C phage.

The requirement, by the extract, for divalent cations for phage inactivation is similar to that for effective attachment of phage to host cells. Sodium ions proved inhibitory to extract inactivation of the phage; they also inhibit phage attachment. The PG broth used to assay the effects of cations showed a considerable reduction in the plaque-forming capacity of phage controls when compared to Ca- and Mg-supplemented diluents (Table 5). The reduction in PFU/ml in the PG broth and in Na- and K-supplemented diluents is attributed to a reduction in the ability of the phage to attach to host cells. The addition of extract produced very little phage inactivation when compared to the phage control for each diluent. In the Ca- and Mg-supplemented diluents, the phage control values and per cent inactivation by the extract were comparable to those obtained in PY broth.

The sensitivity of the inactivating complex to proteolytic enzymes, 2-mercaptoethanol, and thymol implies that the inactivating substance contains a protein of some complexity. While the detergents may affect the proteins, the activity of Tween 80 and sodium lauryl sulfate on the extract and the resistance of the extract to boiling indicate that lipids or carbohydrates, or both, may be associated with the protein(s). Preliminary thin-layer chromatography of extracts developed with diisobutylketone-acetic acid-water (40:25:5) has shown the presence of phosphatidyl ethanolamine and several classes of phospholipids and lipoproteins. Phosphatidyl ethanolamine proved to have no effect on the viability of phage suspensions.

Although proteolytic enzymes and several other chemical agents were effective in destroying the inhibitory substance, it was not possible to reverse the effects of phage inactivation with any of the agents. Thus, once the phage are inactivated, a chemical alteration of the inactivating complex does not leave phage free for attachment or infection. One explanation for this observation is that the site of phage and inhibitor binding is not affected by the chemical agents, but, rather, secondary sites are affected. Such an alteration may prevent phage and extract from binding, but, once binding takes place, could not reverse the effects, because chemical residues remain attached to the phage. Another explanation is that the inactivation of the phage results in the release of phage deoxyribonucleic acid.

ACKNOWLEDGMENTS

This investigation was supported by a Brown-Hazen Grant of the Research Corporation and by institutional support from the Medical College of Georgia.

LITERATURE CITED

1. Adams, J. N. 1964. Recombination between *Nocardia erythropolis* and *Nocardia canicruria*. J. Bacteriol. 88:865-876.
2. Adams, M. H. 1959. Bacteriophages. Academic Press Inc., New York.
3. Adams, M. M., J. N. Adams, and G. H. Brownell. 1970. The identification of *Jensenia canicruria* Bisset and Moore as a mating type of *Nocardia erythropolis* (Gray and Thornton) Waksman and Henrici. Int. J. Syst. Bacteriol. 20:133-147.
4. Brownell, G. H., and J. N. Adams. 1968. Linkage and segregation of a mating type specific phage and resistance characters in nocardial recombinants. Genetics 60:437-448.
5. Brownell, G. H., J. N. Adams, and S. G. Bradley. 1967. Growth and characterization of nocardio-phage for *Nocardia canicruria* and *Nocardia erythropolis* mating types. J. Gen. Microbiol. 47:247-256.
6. Groman, N. B., and K. McCormick. 1961. Relation between adsorption of diphtheria phage and its inactivation by an oleic acid-activated inhibitor. J. Bacteriol. 81:394-400.
7. Sellers, M. I., and T. Tokunaga. 1970. Inactivation of mycobacteriophages by lipid solvents. Host-virus relationships in *Mycobacterium*, *Nocardia*, and *Actinomyces*, p. 134-143. In S. E. Juhasz and G. Plummer (ed.), Proceedings of a symposium. Charles C Thomas, Publisher, Springfield, Ill.
8. Tokunaga, T., T. Kataoka, and K. Suga. 1970. Phage inactivation by an ethanol-ether extract of *Mycobacterium smegmatis*. Amer. Rev. Resp. Dis. 101:309-313.
9. Tokunaga, T., M. I. Sellers, and A. Furuchi. 1970. Phage receptor of *Mycobacterium smegmatis*. Host-virus relationships in *Mycobacterium*, *Nocardia*, and *Actinomyces*, p. 119-133. In S. E. Juhasz and G. Plummer (ed.), Proceedings of a symposium. Charles C Thomas, Publisher, Springfield, Ill.
10. Weidel, W. 1958. Bacterial viruses. Annu. Rev. Microbiol. 12:27-48.