Growth and Characterization of Nocardiphages for *Nocardia canicruria* and *Nocardia erythropolis* Mating Types

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SUMMARY

Two nocardiphages were isolated from soil samples: one, called ϕC , was specific for the *Nocardia canicruria* mating type; the other, called ϕEC , proliferated in *N. canicruria* and *N. erythropolis* mating types. Inactivation of phage ϕC was rapid in NaCl solutions, with chloroform, 0.3% hydrogen peroxide and 30% (v/v) ethanol in water. Phage ϕEC behaved similarly but was less labile in NaCl solutions. Both phages were resistant to diethyl ether. Phage ϕC could be propagated to titres of 10^{10} plaque-forming units/ml. on a medium containing peptone, yeast extract and calcium nitrate; the highest observed titres of phage ϕEC were also produced in this medium. A chemically defined medium for proliferation of phage ϕC consisted of inorganic salts, valine, isoleucine, leucine, nicotinamide, glycerol. Other variables which affected the growth of these phages were the amount of phage and host inocula and the age of the host before infection. Phage ϕC was distinct from other nocardiphages in its ability to attach quickly and efficiently to its host; 95% of the phage attached, and attachment was time and temperature dependent. Attachment of phage ϕEC was slow and inefficient, reaching only 34%. The latent period for phage ϕC was about 25 min., the increase period was 35 min. and the burst size about 60 particles/infective centre. The latent period of phage ϕEC was 180 min. and the burst size about 20 particles. Both these nocardiphages possessed the typical actinophage morphology.

INTRODUCTION

Genetic recombination between nocardias of heterologous origin has been demonstrated (Adams, 1964). In general, substrains derived from *Nocardia erythropolis* do not recombine with each other nor will substrains derived from *N. canicruria*, but 'interspecific' combinations are fertile. These observations indicate a mating factor(s) governing compatibility in nocardial recombination. In further investigations of hereditary phenomena in nocardias, characters have been sought for use in the development of linkage maps. Specific phage-resistance characters are deemed appropriate for such purposes.

Two nocardiphages were isolated from soil. One (phage ϕC) specifically infected the *N. canicruria* mating type while the second (phage ϕEC) infected both the *N. canicruria* and *N. erythropolis* mating types. Although mating-type specific phages have been described for *Escherichia coli* (Loeb, 1960; Dettori, Maccacaro & Piccinin,

1961), they are largely unknown for other bacterial systems. To exploit nocardiphages as genetic markers and to ascertain their exact relationships to mating type, it has been necessary to grow and characterize them. In this report, the conditions necessary for propagating and assaying two nocardiphages are defined, thereby allowing for their further characterization. Special attention has been given phage ϕ C because of its potential application in studies of compatibility phenomena in the nocardias.

METHODS

Cultures. The hosts used were *Nocardia canicruria* strain 3 (Adams, 1964) and *N. canicruria* strain 57 (Anderson & Bradley, 1961). These strains were originally designated *Jensenia canicruria* (Bisset & Moore, 1950). *Nocardia erythropolis* strain 2 (Adams, 1964) was also used. Stock cultures were maintained on nutrient agar (Difco) or on peptone + yeast extract (Difco) medium (PY) composed of 0.5% (w/v) peptone and 0.3% (w/v) yeast extract solidified, when necessary, with 1.5% (w/v) agar. Cultures were maintained at 30°.

Plaque assay medium and phage propagation medium. The plaque-forming capacities of lysates containing phage ϕ C or phage ϕ EC were determined by the conventional soft-agar overlay technique (Jones & Bradley, 1962) with nutrient agar or PY agar as the nutrient. In some tests, the assay media were supplemented with 0.01 M-NaCl, 0.1 M-NaCl, or 0.01 M-Ca(NO₃)₂. Plaque numbers were not appreciably affected by supplementation and PY agar containing 0.01 M-Ca(NO₃)₂ was arbitrarily chosen as the routine plaque assay medium. To determine the effects of salts on nocardiphage growth, nutrient broth or PY broth supplemented with NaCl or Ca(NO₃)₂ was inoculated with *Nocardia canicruria* to give an extinction of 0.1 at 620 m μ . After incubation for 2 hr at 30°, phage ϕ C or phage ϕ EC was added to a final concentration of 1×10^7 plaque-forming units (p.f.u.)/ml. The phage-infected cultures were assayed for increase in p.f.u. values after incubation for 4 or 12 hr.

Determination of nocardiphage stability. To discover suitable diluents for phages ϕ C and ϕ EC, 0.1 ml. samples of stock phage preparations were added to various diluents and maintained at 25° for 2 hr. The stability of phages ϕ C and ϕ EC to materials commonly lethal for other bacteriophages was tested by incubating mixtures of nocardiphage with different reagents at 25° for 2 hr.

Determination of factors which affect nocardiphage attachment. Several factors were tested for their effect upon attachment of nocardiphage. A 2 hr culture of *Nocardia canicruria* was harvested and resuspended in different media to an extinction of 0.4 at 620 m μ . Phage was added and 5 min. later the mixture was sampled, diluted into 4 volumes of broth, centrifuged and the titre of the supernatant fluid determined. Attachment was expressed as the ratio of free phage particles present in the supernatant fluid to the total phage count. Alternatively, a sample of the whole culture was shaken with an equal volume of diethyl ether and the titre of the aqueous layer measured. In this case, attachment was expressed as the ratio of the number of phage particles present in the aqueous phase of the ether-treated culture to that of the untreated sample. Another method used to measure phage to host attachment involved the addition of specific antiphage serum (Bradley, Papermaster, Watson & Good, 1961). Phage able to form plaques after the addition of antiserum was considered to be attached to the host.

Effect of host age and inoculum sizes on phage propagation. The effect of host age on phage propagation was tested by adding suitable concentrations of phage to the host which had been incubated for various times. Twelve hr after the addition of phage, the phage titre was determined. The effect of the size of the phage inoculum on propagation was examined by mixing phage inocula at different concentrations with a standard host concentration and measuring the p.f.u. after incubation for 3 hr and 24 hr. The effect of the host-inoculum size on phage proliferation was determined by varying the host inoculum concentration and assaying plaque numbers.

Propagation dynamics. A 2 hr host culture having an extinction of 0.1 at 620 m μ in PY broth supplemented with 0.01 M-Ca(NO₃)₂ was inoculated with phage ϕ C, 5×10^8 p.f.u./ml. or phage ϕ EC, 1.9×10^6 p.f.u./ml. At intervals, after allowing 10 min. for adsorption, samples were removed, diluted in fresh medium and assayed for plaque numbers.

Minimal medium. A 3 hr culture of *Nocardia canicruria* was harvested and resuspended in 0.001 M-Ca(NO₃)₂ (pH 7) to an extinction of 0.2 at 620 m μ . To this suspension various supplements were added and phage ϕ C added to obtain 2.5×10^8 p.f.u./ml. in the mixture. After incubation for 3 hr at 30°, the mixtures were assayed for plaque numbers.

Host range. The host ranges of phages ϕ C and ϕ EC were determined using methods described previously (Anderson & Bradley, 1961). The host ranges of ϕ C and ϕ EC were compared to the host range of phage MJP 1. This phage, MJP 1, was isolated on *Nocardia canicruria* and its host range previously described (Anderson & Bradley, 1961).

Morphology. The structural features of phages ϕ C and ϕ EC were determined by examining negatively-stained preparations with a Siemen's electron microscope according to the methods of Painter & Bradley (1965).

RESULTS

Although the nutrient and salt composition of the plaque assay medium did not markedly affect the titres of phages ϕ C and ϕ EC, the composition of the propagation medium was critical. Peptone+yeast extract broth was the best routine nutrient source (Table 1). The addition of Ca(NO₃)₂ stimulated phage production but 0.1 M-NaCl was deleterious. The highest routine phage titres were obtained in PY broth supplemented with 0.01 M-Ca(NO₃)₂.

Phage ϕ C was rapidly inactivated in many salt solutions (Table 2); it was susceptible to M-NaCl, but was stable for 2 hr at 25° in 0.01 M-Ca(NO₃)₂, MgSO₄ or tris buffer, and in nutrient broth or PY broth at pH 7. Phage ϕ EC followed the stability pattern of phage ϕ C except that ϕ EC was somewhat more stable in NaCl solutions.

The two phages were similar in their patterns of resistance to deleterious agents (Table 3). Chloroform and thymol (frequently used to render phage lysates free from living bacteria) decreased the titre of phage ϕ C 10,000-fold; chloroform was somewhat less lethal to phage ϕ EC, decreasing the titre only 100-fold. Both phages were rapidly inactivated by ultraviolet irradiation.

Table 1. *Complex medium for propagation of nocardiphages ϕC and ϕEC*

Nocardia canicruria, at an extinction of 0.1 at 620 m μ , was incubated for 2 hr at 30°. After host incubation, phages ϕC or ϕEC were added to give a final phage concentration of 10⁷ p.f.u./ml. After 4 or 12 hr further incubation, plaque numbers were determined.

Medium	Supplement	ϕC (p.f.u./ml.)		ϕEC (p.f.u./ml.)
		4 hr	12 hr	12 hr
Nutrient broth	—	2.0 $\times$ 10 ⁷	3.3 $\times$ 10 ⁸	2.0 $\times$ 10 ⁹
	0.1 M-NaCl	< 1 $\times$ 10 ⁵	< 1 $\times$ 10 ⁵	3.0 $\times$ 10 ⁸
	0.01 M-NaCl	1.8 $\times$ 10 ⁷	4.4 $\times$ 10 ⁸	3.0 $\times$ 10 ⁹
	0.01 M-Ca(NO ₃) ₂	3.5 $\times$ 10 ⁹	5.3 $\times$ 10 ⁹	1.8 $\times$ 10 ⁸
	0.001 M-Ca(NO ₃) ₂	3.9 $\times$ 10 ⁹	6.4 $\times$ 10 ⁹	6.9 $\times$ 10 ⁹
PY broth	—	4.4 $\times$ 10 ⁸	5.1 $\times$ 10 ⁹	2.3 $\times$ 10 ⁹
	0.1 M-NaCl	< 1 $\times$ 10 ⁵	< 1 $\times$ 10 ⁵	1.0 $\times$ 10 ⁴
	0.01 M-NaCl	4.7 $\times$ 10 ⁸	3.9 $\times$ 10 ⁹	9.0 $\times$ 10 ⁷
	0.01 M-Ca(NO ₃) ₂	8.2 $\times$ 10 ⁹	1.6 $\times$ 10 ¹⁰	3.3 $\times$ 10 ⁹
	0.001 M-Ca(NO ₃) ₂	1.6 $\times$ 10 ¹⁰	9.0 $\times$ 10 ⁹	2.4 $\times$ 10 ⁹

Table 2. *Diluents for nocardiphages ϕC and ϕEC*

A 0.1 ml. sample of stock preparations of phages ϕC or ϕEC was added to 10 ml. of the diluent to be tested. All solutions were previously adjusted to pH 7.0. After 2 hr incubation at 25°, the diluent-phage mixtures were assayed for viable phage.

Diluent	Phage ϕC (p.f.u./ml.)	Phage ϕEC (p.f.u./ml.)
Deionized water	9.9 $\times$ 10 ⁵	9.4 $\times$ 10 ⁵
NaCl (0.001 to 0.1 M)	8.6 $\times$ 10 ⁵	1.2 $\times$ 10 ⁶
NaCl (M)	1.0 $\times$ 10 ²	6.7 $\times$ 10 ⁵
PY broth	2.3 $\times$ 10 ^{6*}	3.1 $\times$ 10 ^{6*}
Nutrient broth	3.3 $\times$ 10 ^{6*}	3.7 $\times$ 10 ^{6*}
MgSO ₄ (0.001–0.01 M)	2.7 $\times$ 10 ^{6*}	—
Ca(NO ₃) ₂ (0.001–0.01 M)	2.4 $\times$ 10 ^{6*}	1.1 $\times$ 10 ^{6*}
KH ₂ PO ₄ + K ₂ HPO ₄ (0.1 M)	5.1 $\times$ 10 ³	—
KH ₂ PO ₄ + K ₂ HPO ₄ (0.01 M)	5.3 $\times$ 10 ⁵	—
Tris (0.01 M)	2.9 $\times$ 10 ^{6*}	—
0.01 M-tris in 0.001 M-Ca(NO ₃) ₂	2.5 $\times$ 10 ^{6*}	—
0.1 M-NaCl + 0.001 M-Ca(NO ₃) ₂	2.6 $\times$ 10 ^{6*}	1.1 $\times$ 10 ^{6*}

* Satisfactory diluent. —, Not done.

Nocardiphage attachment

Attachment of phage ϕC was markedly affected by medium composition (Table 4). When water was used as the attachment medium, only 5% of phage ϕC particles were effectively attached; the addition of 0.01 M-Ca²⁺ to PY broth facilitated its attachment. Other media tested were less satisfactory for attachment.

Three methods were used to determine attachment (Table 5). The methods based on the use of antisera or ether sensitivity were equally useful, and indicated slightly greater degrees of attachment than did the centrifugation method.

Attachment of phage ϕC was retarded at 5° (Table 6). The best attachment of phage ϕC was at the highest temperature tested (37°). In contrast, phage ϕEC attached inefficiently; for example, after 30 min. incubation at 30° in PY broth containing 0.01 M-Ca²⁺, phage ϕC attached with an efficiency of 95%, whereas phage ϕEC attached with an efficiency of 34%.

Table 3. *Effects of chemical and physical agents on nocardiphages ϕC and ϕEC*

To determine the effects of physical treatments, PY broth grown preparations of phages ϕC or ϕEC were diluted 1 to 10 in PY broth. A 0.1 ml. sample was added to 10 ml. PY broth, the physical treatment applied, and viable phage numbers were estimated immediately after the treatment. A similarly diluted but untreated PY broth preparation served as a control. To determine the effects of the chemical agents, PY broth grown preparations of the phages were diluted 1 to 10 in water. A 0.1 ml. sample of the dilution was added to 10 ml. of the reagent to be tested at the specified concentration in water. After 2 hr incubation at 25°, the mixtures were assayed for phage numbers. *Nocardia canicruria* was used as the host in all cases.

Treatment	Phage ϕC (p.f.u./ml.)	Phage ϕEC (p.f.u./ml.)
Untreated control	2.7×10^8	3.7×10^8
Chloroform*	1.2×10^4	1.8×10^4
Thymol (0.1 g./added to 10 ml. water)	1.0×10^8	—
Diethyl ether†	1.6×10^8	3.2×10^8
Hydrogen peroxide (3 %, v/v, in water)	$< 1 \times 10^3$	$< 1 \times 10^3$
Hydrogen peroxide (0.3 %, v/v, in water)	1.6×10^4	1.5×10^3
Mercaptoethanol (1.5 M in water)	9.0×10^4	5.0×10^5
Mercaptoethanol (0.15 M in water)	9.4×10^7	1.0×10^6
Ethanol (30 %, v/v, in water)	1.0×10^8	$< 1 \times 10^3$
Freezing	2.7×10^8	3.1×10^8
45° for 15 min.	1.7×10^7	4.2×10^4
56° for 15 min.	8.0×10^3	1.0×10^3
Ultrasonic treatment (20 kc./sec.) for 60 sec.	2.4×10^6	—
French press (4000 lb./sq.in.)	1.7×10^6	—
Ultraviolet irradiation for	30 sec.	1.0×10^4
	90 sec.	1.7×10^3
	150 sec.	4.1×10^3
	210 sec.	$< 10^1$

* Biphasic: 1 vol. chloroform to 2 vol. water. † Biphasic: 1 vol. ether to 3 vol. water.
—, Not done.

Table 4. *Total attachment of nocardiphage ϕC to washed *Nocardia canicruria* organisms*

A 2 hr culture of *Nocardia canicruria* was harvested and resuspended in the indicated media to an extinction of 0.4 at 620 m μ . Phage was added and after 5 min. to allow for attachment, the mixture was sampled, diluted into 4 vol. of broth, centrifuged and the viable titre of the supernatant fluid was assayed. Alternatively, a whole culture and a sample were shaken with an equal volume of diethyl ether and the phage titre of the aqueous layer was determined.

Attachment medium	Titre $\times 10^{-3}$			
	Total phage ϕC	free phage ϕC	% attached	% ether- sensitive
Water	339	321	5	11
0.1 M-NaCl	96	50	48	50
Nutrient broth	295	138	53	62
0.001 M-Ca(NO ₃) ₂	255	108	58	63
0.01 M-Ca(NO ₃) ₂	281	101	64	63
PY broth	268	115	57	68
PY broth + 0.01 M-Ca(NO ₃) ₂	248	24	90	93
0.1 M-NaCl + 0.1 M-Ca(NO ₃) ₂	170	88	48	56

Table 5. *Comparison of three criteria for measuring attachment of nocardiphage ϕ C to Nocardia canicruria cells*

A 2 hr culture of *Nocardia canicruria* was harvested and resuspended in the indicated media to an extinction of 0.4 at 620 m μ . Phage ϕ C was added to a concentration of 1.9×10^7 p.f.u./ml. After 10 min., attachment was determined by the indicated criteria.

Attachment index	Attachment medium	
	0.003 M-Ca(NO ₃) ₂	Peptone+yeast extract medium+0.01 M-Ca(NO ₃) ₂
	% phage attached in	
Ether sensitivity	88.5	99.3
Antiserum resistant	92.1	100
Free phage	80.6	92.4

Table 6. *Effects of time and temperature on attachment of nocardiphage ϕ C to Nocardia canicruria*

A culture was equilibrated to the indicated temperature and phage ϕ C added to a final concentration of 3.3×10^8 p.f.u./ml. At the indicated times, 1 ml. samples were mixed with 1 ml. of diethyl ether; the aqueous layer was assayed for viable phage.

Time (min.)	Temperature		
	5°	30°	37°
	Free phage (temperature variable)		
0+	3.3×10^8	1.3×10^8	4.8×10^5
2	5.6×10^8	1.0×10^8	1.8×10^5
4	4.3×10^8	8.5×10^5	1.5×10^4
6	2.9×10^8	1.3×10^5	1.1×10^4
8	2.4×10^8	2.0×10^5	6.0×10^3
10	2.6×10^8	2.1×10^5	2.5×10^3

Nocardiphage propagation

From the results of the preceding experiments, it was evident that several factors affected the phage to host interaction. When host age before infection was examined, it was found (Table 7) that young cultures gave the best yields of phage. However, the time at which the phage+host mixture (Table 8) was sampled also determined phage yield. In a 3 hr period, the phage output was directly related to the size of the phage inoculum. With an 18 hr incubation time, the final phage yields were practically identical regardless of the multiplicity of infection. Upon varying the amount of the host (Table 9), an increased yield was observed with an increase of host. These data indicate that a maximum yield of phage was dependent upon the absolute number of host organisms, the growth stage of the host, and ratio of phage to host.

Under standard conditions, with young host cultures, the growth curves of phages ϕ C and ϕ EC were distinct from each other, but typical of phage growth curves. The latent period for phage ϕ C was about 20 min., the increase period was 40 min. and the burst size about 60 particles/infective centre. Phage ϕ EC had a latent period extending to 180 min. and the burst size was lower than that for ϕ C, approaching only 20 particles/infective centre.

Table 7. *Effect of host age on production of nocardiphages ϕC and ϕEC*

Peptone+yeast extract medium was inoculated with *Nocardia canicruria* to an initial population density of 5×10^7 cells/ml. At intervals, phage was added to a final concentration of 2×10^6 p.f.u./ml. and the mixture incubated for 12 hr. After incubation, the cultures were centrifuged and the supernatant fluid assayed for plaque numbers.

Phage	Host	Time before phage added (hr)	Titre
ϕC	<i>N. canicruria</i>	0	6.0×10^8
	<i>N. canicruria</i>	3	1.5×10^9
	<i>N. canicruria</i>	5	2.2×10^8
	<i>N. canicruria</i>	12	$< 1 \times 10^7$
ϕEC	<i>N. canicruria</i>	0	1.0×10^9
	<i>N. canicruria</i>	3	2.0×10^9
	<i>N. canicruria</i>	12	$< 1 \times 10^7$
	<i>N. erythropolis</i>	0	7.1×10^7
	<i>N. erythropolis</i>	3	7.5×10^8
	<i>N. erythropolis</i>	12	$< 1 \times 10^8$

Table 8. *Effect of size of nocardiphage ϕC inoculum on yield*

A 2 l. flask containing 500 ml. of peptone+yeast extract+0.01 M-Ca(NO₃)₂ broth was inoculated with 10 ml. of an 18 hr. culture of *Nocardia canicruria*. The host was incubated for 2 hr before phage ϕC was added at the indicated concentrations. After incubation for 3 or 18 hr the cultures were assayed for plaque numbers.

Phage inoculum size (p.f.u./ml.)	Output of phage at	
	3 hr	18 hr
	(p.f.u./ml.)	
10^8	5×10^9	3×10^9
10^7	3×10^9	5×10^9
10^6	3×10^8	9×10^9
10^5	2×10^7	9×10^9
10^4	4×10^6	1.8×10^{10}
10^3	3×10^5	1.6×10^{10}
10^2	1×10^5	1.1×10^{10}
10^1	1×10^5	1.1×10^{10}

Table 9. *Effect of size of the host inoculum on proliferation of nocardiphage ϕC*

Nocardia canicruria was incubated for 2 hr at 30°, harvested and suspended in fresh medium to various concentrations. After 1 hr incubation at 30°, phage ϕC was added and the mixture incubated for 200 min. at 30°.

Amount of host colony count/ml.	Plaque-forming units/ml.	
	Phage input 3.2×10^3	Phage input 3.2×10^4
	yield (p.f.u./ml.)	
4.3×10^7	6.7×10^5	2.9×10^7
4.3×10^6	2.4×10^4	5.2×10^5
4.3×10^5	7.8×10^3	1.3×10^5
4.3×10^4	2.1×10^2	1.2×10^4

By the method of group and single additions or omissions, a chemically defined propagation medium was devised (Table 10). For maximum production of phage ϕC , a medium composed of glycerol, nicotinamide, basal salts and three amino acids was most effective. The omission of any one of these supplements or groups of supplements (e.g. vitamins) resulted in lowered production of phage. The omission of glycerol, which resulted in a somewhat smaller production of phage, was the least necessary of the defined medium constituents.

Table 10. *Simplified medium for the propagation of nocardiphage ϕC in Nocardia canicruria*

Supplement	Concentration	Phage yield (p.f.u./ml.)
Salts*	1 ml./11 ml.	3.6×10^6
Vitamins†	0.1 ml./11 ml.	
Peptone	5 mg./ml.	1.2×10^{10}
Yeast extract	3 mg./ml.	
Casamino acids	1 mg./ml.	3.6×10^8
Casamino acids	1 mg./ml.	1.3×10^9
Salts	1 ml./11 ml.	
Casamino acids	1 mg./ml.	1.0×10^{10}
Salts	1 ml./12 ml.	
Vitamins	0.1 ml./12 ml.	
Amino acids‡	1 ml./12 ml.	8.1×10^9
Salts	1 ml./12 ml.	
Vitamins	0.1 ml./12 ml.	
Valine	1 ml./12 ml.	3.1×10^9
Salts	1 ml./12 ml.	
Vitamins	0.1 ml./12 ml.	
Isoleucine	1 ml./12 ml.	3.1×10^9
Salts	1 ml./12 ml.	
Vitamins	0.1 ml./12 ml.	
Leucine	1 ml./12 ml.	2.0×10^9
Salts	1 ml./12 ml.	
Vitamins	0.1 ml./12 ml.	
Nicotinamide	0.1 ml./12 ml.	2.2×10^{10}
Salts	1 ml./12 ml.	
Amino acids	1 ml./12 ml.	
Glycerol	0.1 ml./12 ml.	7.0×10^{10}
Nicotinamide	0.1 ml./12 ml.	
Salts	1 ml./12 ml.	
Amino acids	1 ml./12 ml.	

* The salt solution consisted of KNO_3 , 1 g.; K_2HPO_4 , 1 g.; $MgSO_4$, 0.5 g.; distilled water, 100 ml.

† The vitamins consisted of 0.1 g. each of thiamine, pyridoxine, nicotinic acid and riboflavin; distilled water, 20 ml.

‡ The amino acids consisted of 0.1 g. each of isoleucine, leucine and valine; distilled water, 20 ml.

Host range

Nocardiphage ϕC attacked two strains of *Nocardia canicruria* and an organism designated *N. corallina* strain N305. Phage ϕC was unable to lyse any other of the strains tested (Table 11). *N. canicruria*, *N. erythropolis*, *N. opaca* and *N. corallina* strain N305 were lysed by phage ϕEC ; other nocardias tested were not susceptible to

the action of this phage. Thus the range of increasing lytic capability extends from phage ϕ C to ϕ EC to MJP1.

Morphology of nocardio-phages ϕ C and ϕ EC

Negatively stained phage ϕ C was found to have a head 52 ± 2 m μ in diameter and tail 10 m μ wide $\times$ 192 ± 8 m μ long (Pl. 1, A). The head size of phage ϕ EC (Pl. 1, B) was 52 m μ in diameter and the tail 10 m μ wide $\times$ 197 m μ long.

Table 11. *Host ranges of nocardio-phages ϕ C, ϕ EC and MJP1*

Stock PY preparations of the phages, containing ca. 1×10^9 p.f.u./ml., were diluted for three ten-fold serial dilutions. 0.1 ml. samples of the dilutions were added to ca. 5×10^7 host cells/ml. contained in 2.5 ml. soft overlay agar at 45°. The phage-host mixtures were poured on the surface of PY agar plates. The production of plaques after 24 hr. incubation for any dilution tested was considered as a positive result.

Organism and strain	ϕ C	ϕ EC	MJP1
<i>Nocardia canicruria</i> 1574	+	+	+
<i>N. canicruria</i> 57	+	+	+
<i>N. corallina</i> 2747	—	—	—
<i>N. corallina</i> w5	—	—	—
<i>N. corallina</i> 107	—	—	—
<i>N. corallina</i>	—	—	nt
<i>N. corallina</i> w3408	—	—	nt
<i>N. corallina</i> 78	—	—	—
<i>N. corallina</i> s5	—	—	—
<i>N. corallina</i> N305	+	+	+
<i>N. brasiliensis</i> 301	—	—	+
<i>N. opaca</i> 76	—	—	—
<i>N. opaca</i> 765A	—	+	nt
<i>N. erthropolis</i>	—	+	+
<i>N. farcinica</i>	—	—	nt
<i>N. rubra</i> 1	—	—	—
<i>N. rubra</i> 74	—	—	—
<i>N. asteroides</i> 58	—	—	nt
<i>N. asteroides</i> 133	—	—	nt
<i>N. asteroides</i> 300	—	—	nt
<i>N. asteroides</i> 162	—	—	nt
<i>N. asteroides</i> 92	—	—	nt
<i>N. rhodnii</i> N 6117	—	—	nt
<i>Mycobacterium rhodochrous</i> 370	—	—	—
<i>M. rhodochrous</i> 482	—	—	—

nt: not tested.

DISCUSSION

Nocardiophage ϕ C was routinely propagated to titres of 10^{10} p.f.u./ml. in this work. Attempts to purify the phage further by centrifugation or column chromatographic separation procedures were unsuccessful; phage ϕ C was sensitive to these processes. Inactivation under these conditions apparently resulted from damage to the long, delicate phage tail. Nocardiophage ϕ EC is similar to ϕ C in susceptibility to chemical and physical agents; but ϕ EC is easily concentrated by centrifugation without loss of titre.

Unlike phage ϕ EC and other actinophages, phage ϕ C attached efficiently to its host. The observed attachment was irreversible and dependent upon time and

temperature. Attachment proceeded best in a nutrient medium; phages ϕ C and ϕ EC both proliferated in a peptone+yeast extract broth medium containing 0.001 M- $\text{Ca}(\text{NO}_3)_2$. These results suggest that either organic cofactors or host metabolism are required for attachment. The composition of the defined medium for phage ϕ C propagation indicated that proliferation was not solely a question of host nutrition because the host, *Nocardia canicruria*, is easily grown in a simple defined glucose+basal salts medium (Adams, 1964). Nocardiphage ϕ EC also resembles other actinophages more closely than does ϕ C with respect to their latent periods.

Host populations which had been incubated for more than about 5 hr before infection with phage ϕ C or ϕ EC gave low yields of phage. These results may be correlated with the developmental cycles of the host (Adams & McClung, 1962; Adams, 1964). The interval from 0 to 5 hr appears to represent the lag and germination phase of *Nocardia canicruria*. From the present work, it appears that the host is most susceptible to viral infection of these phages ϕ C and ϕ EC during this period. With further growth after incubation for 5 hr, a well-defined coenocytic development stage of the nocardias was noted. Multiple attachment of phage particles to the filaments may account for the decrease in nocardiphage proliferation. Unfortunately, unlike the Eubacteriales, synchronous growth of the actinomycetes in general has not been successfully achieved; only under conditions of synchronous growth of the host organisms will the more subtle of the nocardiphage+host interactions be easily explored.

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EXPLANATION OF PLATE

Electron micrographs of nocardiphages ϕ C (A) and ϕ EC (B). The marker bars denote 250 m μ .