Isolation and Partial Characterization of Some New Bacteriophages Active Against *Asticcacaulis* Strains

CAROL A. MIDDLETON AND J. L. PATE

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

The isolation and properties of 42 phages active against *Asticcacaulis* strains AC2, C19, AC12, AC48, S-1, S-3, and KA4A are described. The phages are divided into 12 groups according to host range and serology. Phages in groups I through IX are lytic for strain AC48 but not for AC2 or C19; those in groups I through VIII are also lytic for strains S-1 and S-3. Phages in groups X through XII are lytic only for strains AC2 and C19. Six *Caulobacter* strains are sensitive to one or more of these phages. The phages have no activity on strains of the 54 bacterial species, other than caulobacters, tested. Taxonomic implications are discussed. Mitomycin C treatment of the *Asticcacaulis* strains failed to induce any temperate phages but did induce three lytic activities.

Asticcacaulis and *Caulobacter* are members of the family *Caulobacteraceae*, which comprises gram-negative, unicellular, prosthecae bacteria that multiply by transverse binary fission (7, 13). These organisms undergo a dimorphic life cycle, during which a nonmotile prosthecae cell with a secreted holdfast divides to give rise to a motile, flagellated cell. The motile cell produces a holdfast, loses the flagellum, and differentiates a prosthecae to complete the cycle. Known strains of *Asticcacaulis* have a rod-shaped cell with either subpolar prosthecae and flagellum or with two lateral prosthecae and a polar flagellum (12, 13). Holdfast material is produced at or near the pole of the *Asticcacaulis* cell at a site different from that at which prosthecae develop.

Although numerous phages have been isolated which are lytic for species of *Caulobacter* (14; Szezyk and Gerencser, *Bacteriol. Proc.*, 1967), only five phages have been isolated for *Asticcacaulis*. Of these five, ϕ Ac20, 21, and 22 are identical according to their host range and antiserum neutralization characteristics, and ϕ Ac24 and 25 are similar if not identical (14; J. M. Schmidt, personal communication). Temperate phages have also been demonstrated in *Caulobacter* strains (6), but they have not been seen in *Asticcacaulis* (6, 14).

Caulobacter phages have been used as a source of messenger ribonucleic acid (11), to mediate gene transfer (J. M. Schmidt and B. Ely, personal communications), to clarify the taxonomy of caulobacters (13, 14; Szezyk and Gerencser, *Bacteriol. Proc.*, 1967), to isolate phage-resistant mutants for genetic studies (9, 10), and to assay morphogenesis in synchronous populations of caulobacters (3, 8, 15-17).

The present report deals with the isolation and partial characterization of bacteriophages capable of infecting various strains of *Asticcacaulis*. This study started as an attempt to isolate a viral system capable of mediating gene transfer in *Asticcacaulis* and to further elucidate the relationship between members of the family *Caulobacteraceae* as well as between caulobacters and members of other families of bacteria.

MATERIALS AND METHODS

Media. The standard complex PYE medium of Poindexter (13) was used for cultivation of *Caulobacter* and *Asticcacaulis* strains. Isolation and propagation of phages were carried out in PYE containing 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Nutrient broth (Difco), TYEg, or 0.5% peptone (Difco) in distilled water was used to propagate other bacterial species used in the host range study. The TYEg medium consisted of 1% tryptone (Difco), 0.3% yeast extract (Difco), and 0.5% glucose in distilled water. The plating medium contained 1.5% agar, and soft-agar overlays contained 0.7% agar. Each plate contained 30 ml of bottom PYE agar.

Bacterial strains. All *Caulobacter* strains as well as *Asticcacaulis* strains AC2, AC12, AC48, S-1, and KA4A were obtained from Jean M. Schmidt of Arizona State University. The designations of these strains are according to Poindexter (13). *A. biprosthecae* C19 (= ATCC 27554) and *Asticcacaulis* S-3 were obtained from our laboratory's culture collection. Strains of the following species were also studied: 11 species of *Bacillus*, *Escherichia coli* (3 strains), *Salmonella enteritidis*, *Citrobacter freundii*, 3 species of *Enterobacter*, *Serratia liquefaciens*, *Serratia marcescens*, *Pectobacterium caratovororum*, *Proteus vulgaris*, *Providencia alcalifaciens*, 4 species of *Micrococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acetobacter aceti*, *Acetobacter suboxydans*, *Agrobacterium radiobacter*, *Agrobacter-*

ium tumefaciens, *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Arthrobacter crystallopoietes*, *Azotobacter vinelandii*, *Brevibacterium linens*, *Chromobacterium lividum*, *Propionibacterium shermanii*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Zooglea ramigera*, 5 species of *Lactobacillus*, *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Streptococcus lactis*, *Mycobacterium smegmatis*, *Nocardia corallina*, *Streptomyces mediterranei*, and *Sphaerotilus* sp. All cultures of these strains were obtained from the University of Wisconsin Department of Bacteriology culture collection.

Isolation of bacteriophages. The enrichment and isolation techniques of Schmidt and Stanier (14) were used for the isolation of bacteriophages from sewage and lake water. This procedure was modified in isolating phages for the slow-growing *A. biprosthecum*. Samples were filtered with a sterile, 0.45- μ m membrane filter (Millipore Corp.) to eliminate competing bacteria and were added to equal volumes of double-strength broth cultures of *A. biprosthecum*. These enrichments were incubated with slow shaking for 24 h at 30 C, were again filtered, and were then spotted on overlays containing the bacterial host. Plates were examined for plaques after 2 and 5 days of incubation at 30 C.

Phage assays were carried out by the agar layer method of Adams (1) using 2 ml of PYE agar, 0.1 to 1.0 ml of phage, and 0.2 to 0.4 ml of an exponentially growing culture of an *Asticcacaulis* strain. The amount of the bacterial inoculum depended on the turbidity of the cultures and was increased to 0.5 ml for *A. biprosthecum*. Plates were incubated at 30 C for 5 days.

Preparation of lysates. Phage stocks were prepared by eluting agar overlayer plates giving confluent lysis. Up to 10 5-min elutions were made with 1.5 ml of PYE broth per plate for each elution. These lysates were filtered or poured over chloroform and stored at 4 C in screw-capped tubes. Titers of 10^7 to 10^{11} plaque-forming units (PFU) per ml were obtained by this method.

Host range determinations. The host range of each phage was determined by spotting a lysate containing 10^7 to 10^{10} PFU on agar overlays of the bacterial strains to be tested. Each plate was spotted with 4 to 10 lysates according to a grid drawn on the bottom of the plate. Plates were incubated at 30 C and were examined for clearing of the lawn after 3 to 5 days for caulobacters and after 24 h for all other organisms. In most cases, no attempts were made to determine whether secondary propagations could be made from primary cleared areas. Thus, the host range that we report might, in some cases, indicate only that the host is sensitive to the phage but does not allow the phage to propagate.

Electron microscopy. Filtered phage lysates were concentrated by ultracentrifugation for electron microscope examination. The lysates were centrifuged for 1 h at $100,000 \times g$ (5 to 15 C) in a Beckman L2-65b ultracentrifuge with a Ti50 rotor head. Pellets were left in the centrifuge tubes overnight at 4 C with 0.2 ml of buffer for slow resuspension.

These phage suspensions were negatively stained with 2% uranyl acetate or with 1% potassium phos-

photungstate (pH 6.9) plus 0.005% bovine serum albumin (KPT-BSA) (4). Suspensions were placed on 200-mesh copper grids with carbon-coated Parlodion films and were stained for 5 s with freshly filtered stain. Specimens were observed with a Zeiss EM9S-2 microscope operated at 60 kV.

Attempted induction of temperate phages. Two methods were used in an attempt to induce possible temperate phages from all *Asticcacaulis* strains. For the broth method (6), mitomycin C (Sigma Chemical Co., St. Louis) was added to exponential-phase *Asticcacaulis* cultures to give a final concentration of 1.0 μ g/ml. Cultures were incubated for 15 min at 30 C on a rotary shaker. The bacteria were sedimented, suspended in PYE, and incubated for 24 h at 30 C. These cultures were filtered, and the filtrates were spotted on all *Asticcacaulis* lawns. Plates were observed after 3 days of incubation at 30 C for formation of plaques.

The rapid plate method was performed as described by Siddiqui et al. (18). All *Asticcacaulis* cultures were spotted onto PYE plates containing 0.01, 0.1, or 1.0 μ g of mitomycin C per ml. Plaques seen within the spots were picked with sterile toothpicks and stabbed to fresh *Asticcacaulis* lawns.

Antiserum preparation and neutralization tests. Two phages were chosen as immunizing antigens, each phage representing a unique host range. Phage stocks containing at least 10^{10} PFU/ml in PYE were used to immunize female albino rabbits.

One week after the first intraperitoneal injection of 1.0 ml of phage suspension, twice weekly intravenous injections were made for 3 weeks, starting with 0.5 ml and increasing to 1.5 ml by week 3. After test bleeding from the marginal ear vein to determine the titers of neutralizing antibody, the rabbits were bled by cardiac puncture, and the sera were stored at -25 C.

Neutralization tests were performed as described by Adams (1), except that the stocks used had a titer of 3×10^6 PFU/ml instead of 10^7 PFU/ml and incubation was at 30 C.

RESULTS

Isolation of bacteriophages. A total of 42 phages were isolated against four *Asticcacaulis* strains. Table 1 shows the origin of each phage. Only 25 samples of source material had to be tested to obtain phages for *Asticcacaulis* strains S-1, S-3, and AC48, but more than 300 sewage and lake samples had to be screened before phages active against strain C19 were found.

Host range determinations. Based on host range and serology, the 42 *Asticcacaulis* phages fall into 12 groups. Table 2 shows the susceptibilities of *Asticcacaulis* strains to these phage groups. The first nine groups are lytic for strain AC48, and all but group IX are also lytic for S-1 and S-3, but none are lytic for *A. biprosthecum* AC2 or C19. The last three groups are lytic only for *A. biprosthecum* AC2 or C19.

TABLE 1. Sources and original hosts of *Asticcacaulis* phages studied

<i>Asticcacaulis</i> phages	Original host (<i>Asticcacaulis</i> strains)	Source ^a
ϕ Ac11, 12	S-3	Raw sewage no. 1
ϕ Ac13	S-3	Raw sewage no. 2
ϕ Ac14	S-3	Raw sewage no. 3
ϕ Ac15	S-3	Raw sewage no. 4
ϕ Ac31	S-3	Raw sewage no. 5
ϕ Ac37	S-1	Raw sewage no. 5
ϕ Ac39	AC48	Raw sewage no. 5
ϕ Ac33, 38	S-3	Raw sewage no. 6
ϕ Ac35, 36	AC48	Raw sewage no. 6
ϕ Ac41, 42, 45, 46	AC48	Pooled lake water and sewage
ϕ Ac57	AC48	Raw sewage no. 7
ϕ Ac59	AC48	Raw sewage no. 8
ϕ AcS ₁ , S ₂ , S ₃ , S ₄ , M ₁ , M ₂ , M ₃ , M ₄ , M ₅ , ML ₁ , ML ₂ , ML ₃ , ML ₄ , ML ₅ , ML ₆	C19	Creek water
ϕ AcS ₅ , S ₆ , S ₇ , ML ₇ , ML ₈ , ML ₉ , ML ₁₀ , ML ₁₁ , J ₁	C19	Lake water

^a The source of raw-sewage samples 1 through 8 was a direct tap into the Madison sewer lines. Sample 1 was taken on 30 March 1973; 2, on 5 April 1973; 3, on 9 April 1973; 4, on 16 April 1973; 5, on 20 July 1974; 6, on 24 July 1974; 7, on 28 October 1974; and 8, on 7 November 1974. The pooled lake water and sewage came from lakes and ponds in Madison, from Madison sewage, and septic-tank sewage from sources near Madison. The creek water was from Willow Creek, and the lake water from the University Bay of Lake Mendota in Madison.

TABLE 2. Host ranges of phages on *Asticcacaulis* and *Caulobacter* strains^a

Phage groups	<i>Asticcacaulis</i> strains						<i>Caulobacter</i> strains		
	AC2	C19	AC12	AC48	S-1, S-3	KA4A	CB8	CB9b, CB24, CB27, CB28b	CB26b
I. ϕ Ac11, 14	—	—	—	+	+	—	—	—	—
II. ϕ Ac12, 13, 15	i	i	—	+	+	i	+	—	—
III. ϕ Ac31, 33, 38, 39	i	i	i	+	+	i	+	—	i
IV. ϕ Ac35, 36	i	i	i	+	+	i	—	—	—
V. ϕ Ac37	i	i	i	+	+	i	—	—	i
VI. ϕ Ac41, 42	i	—	—	+	+	i	+	+	+
VII. ϕ Ac45, 46	—	—	—	+	+	—	—	—	—
VIII. ϕ Ac57	i	i	—	+	+	i	+	—	—
IX. ϕ Ac59	i	—	—	+	—	—	—	—	—
X. ϕ AcM ₂ , M ₃ , M ₄ , M ₅	+	+	—	—	—	—	—	—	—
XI. ϕ AcS ₁	+	+	i	—	i	—	—	—	—
XII. Other C19 phages	+	+	—	—	—	—	—	—	—

^a +, Clearing of the lawn; —, no clearing; i, faint clearing, appears to inhibit growth but not to cause cell lysis.

The following strains of *Caulobacter* were tested for susceptibility to the phages: *C. fusiformis* CB27 and CB29; type II strains CB24 and CB26b; *C. henricii* CB4, CBR, and CB23b; *C. bacteroides* CB7, CB8, CB9b, CB10b, CB11, and CB11a; *C. vibrioides* subsp. *limonus* CB16b; *C. variabilis* CB17b; type V strain CB28b; *C. intermedius* CB63; *C. crescentus* CB1, CB2, KA3, CB13b1a, and CB15; *C. subvibrioides* CB81; *C. subvibrioides* subsp. *albus* CB88 and CB89; *C. leidy* CB37; *C. vibrioides* CBG, CB18a1, and CB51; as well as CB104, CB115, and CB111E, which have been isolated

but not yet characterized by J. S. Poindexter.

Of these *Caulobacter* strains, only six gave positive results with any of the *Asticcacaulis* phages (Table 2). Only group VI could lyse all six of these strains, and groups I, IV, VII, IX, X, XI, and XII gave neither inhibition nor lysis with any of the *Caulobacter* strains tested.

Strains of 54 species of bacteria other than caulobacters were also tested for susceptibility to these phages. None of these organisms showed any susceptibility to the *Asticcacaulis* phages.

Electron microscopy. All phages in groups I

through V and VII through IX are similar in appearance, with heads hexagonal in outline and measuring 65 nm in diameter and with relatively rigid noncontractile tails 160 to 220 nm in length. Representative phages are shown in Fig. 1 through 4. These phages are often seen attached to filaments approximately 3 nm in diameter (Fig. 2 through 4), which is similar to that of pili, but no further evidence has been sought for their site of attachment. Terminal appendages are sometimes seen at the site of attachment.

Phages in group VI have elongated heads 45 nm in width and 220 to 280 nm in length and have flexible noncontractile tails 320 to 340 nm in length (Fig. 5).

Group X phages (Fig. 9) have elliptical heads measuring 65 nm in diameter (or 50 nm in uranyl acetate) and 85 to 100 nm in length with flexible noncontractile tails 260 to 270 nm in length.

Phage ϕ AcS₁ (group XI) has a large and elongated head with dimensions of 70 by 260 nm as visualized with KPT stain. Empty heads vary from 70 to 85 nm in diameter and range from 260 to 500 nm in length, nearly double the length of the normal (260-nm) heads (Fig. 7). The head appears more slender when stained with uranyl acetate and gives dimensions of 50 by 260 nm (Fig. 8). A few double-length empty heads are also seen in uranyl acetate (Fig. 6). The flexible noncontractile tails are 10 nm in diameter and 280 to 320 nm long and are sometimes seen attached to filaments approximately 3 nm in diameter.

Phages in group XII have not yet been observed microscopically.

Phage plaque morphology. All phages in groups I through IX give plaques that are cloudy to some degree and have diffuse edges. Plaques vary in size from pinpoint to 1.5 mm in diameter (Table 3). Groups X through XII give clear plaques that are characteristically variable in size. Except for ϕ AcJ₁, S₂, and S₃, all phages in the last three groups have the same plaque morphology. ϕ AcJ₁, after repeated transfers, gave larger plaques 2 to 4 mm in diameter. ϕ AcS₂ and S₃ gave smaller plaques with sharp edges. Figure 10 shows plaques representative of each morphological type.

Induction. Neither the broth method nor the rapid plate method was successful in inducing any temperate bacteriophages from the seven strains of *Asticcacaulis* tested. The rapid plate method was successful, though, in inducing a lytic activity, possibly a bacteriocin, that was not transferable by picking from the clear zone with a toothpick. Three induced strains gave

this result. Induced AC2 was lytic on AC48; induced AC48 was lytic on S-1 and S-3; and induced AC12 was lytic on S-1, S-3, and AC48. The lytic agent was not characterized further. No phages or lytic activities were seen in uninduced cultures.

Serology. Antiserum neutralization tests showed complete serological nonrelatedness between the *A. biprosthecum* phage ϕ AcM₂ and any phages in groups I through IX and between ϕ Ac38 and any *A. biprosthecum* phages (groups X, XI, and XII). First-order reaction constants are given in Table 4. K values of less than 1 indicate serological nonrelatedness. Of the *A. biprosthecum* phages (groups X, XI, and XII), ϕ AcM₃ is probably equivalent to ϕ AcM₂, and ϕ AcM₄ and M₅ are probably closely related or equivalent to ϕ AcM₂. All other phages are unrelated to ϕ AcM₂. Phages of groups I through IX all appear to be related to ϕ Ac38, but those of groups X through XII appear to be unrelated to ϕ Ac38. Antiserum to phages other than ϕ AcM₂ or ϕ Ac38 would be needed to further define the relationship between these groups.

Nucleic acid determination. Bradley's acridine orange staining technique (5) was used to determine the type and strandedness of nucleic acid for phages in the first 11 groups. All phages stained with acridine orange gave a bright green color which persisted after treatment with molybdcic or tartaric acid, indicating the presence of double-stranded deoxyribonucleic acid (DNA). Group XII phages were not tested for nucleic acid type.

DISCUSSION

The results of the host range studies have taxonomic implications at the family, genus, and species levels. The *Asticcacaulis* phages showed an absolute specificity for members of the family *Caulobacteraceae* and had no effect on any of the 54 other species tested. This supports Poindexter's argument for the taxonomic uniqueness of the family *Caulobacteraceae* (13) and Schmidt and Stanier's view of caulobacters "as a completely isolated group with respect to host/phage interactions" (14).

However, there is no longer an "... absolute specificity between... [the genera *Asticcacaulis* and *Caulobacter*] with respect to host specificity for bacteriophages," as stated by Schmidt and Stanier (14). They found that none of their *Caulobacter* phages lysed any strains of *Asticcacaulis* and that none of their *Asticcacaulis* phages lysed any *Caulobacter* strains. Eleven of the *Asticcacaulis* phages isolated in this study are capable of lysing from one to six

FIG. 1. Phage ϕ Ac31 stained with uranyl acetate. Head capsomeres and tail striations are apparent. Bar indicates $0.1\ \mu\text{m}$.

FIG. 2. Phage ϕ Ac31 stained with KPT-BSA. Both head and tail have been penetrated by the stain. An electron-transparent ring is evident inside the head. Same magnification as Fig. 1.

FIG. 3. ϕ Ac15 phages attached to a filament $3\ \text{nm}$ in diameter. KPT-BSA stain. Bar indicates $0.2\ \mu\text{m}$.

FIG. 4. Higher magnification of Fig. 3; terminal appendages are apparent. Same magnification as Fig. 1.

FIG. 5. Phage ϕ Ac41 stained with uranyl acetate. Same magnification as Fig. 1.

FIG. 6. Phage ϕ AcS₁ stained with uranyl acetate. One head is empty and nearly double the length of the normal 260-nm heads. Same magnification as Fig. 1.

FIG. 7. Phage ϕ AcS₁ stained with KPT-BSA. Empty capsids of normal (260 nm) and double (500 nm) length are present. Bar indicates 0.1 μ m.

FIG. 8. Phage ϕ AcS₁ stained with uranyl acetate. Same magnification as Fig. 3.

FIG. 9. Phage ϕ AcM₂ stained with uranyl acetate. Same magnification as Fig. 1.

TABLE 3. Phage plaque sizes and morphologies

Phage group	Plaque size (mm)	Plaque morphology
I and II (ϕ Ac11, 12, 13, 14, 15)	~1.5	Very cloudy, diffuse edge
III (ϕ Ac31, 33, 38, 39)	~1.0	Very cloudy, diffuse edge
IV (ϕ Ac35, 36)	~0.5	Cloudy
V (ϕ Ac37)	~1.0	Somewhat cloudy, diffuse edge
VI (ϕ Ac41, 42)	~1.0	Cloudy, diffuse edge
VII (ϕ Ac45, 46)	Pinpoint - 0.5	Cloudy
VIII and IX (ϕ Ac57, 59)	0.8	Cloudy, diffuse edge
ϕ AcJ ₁	2-4	Clear
ϕ AcS ₂ , S ₃	Pinpoint - 1.0	Clear, sharp edge
X, XI, and XII (except ϕ AcJ ₁ , S ₂ , S ₃)	Pinpoint - 2.0	Almost clear

TABLE 4. Antiserum neutralization of *Asticcacaulis* phages

Group	Phage	First-order reaction constants ^a	
		Antise- rum to ϕ AcM ₂	Antise- rum to ϕ Ac38
I	ϕ Ac11	<1	5.85
	ϕ Ac14	<1	6.92
II	ϕ Ac12	<1	5.86
	ϕ Ac13	<1	7.99
	ϕ Ac15	<1	7.18
III	ϕ Ac31	<1	6.75
	ϕ Ac33	<1	7.20
	ϕ Ac38	<1	5.91
	ϕ Ac39	<1	4.30
IV	ϕ Ac35	<1	6.89
	ϕ Ac36	<1	6.44
V	ϕ Ac37	<1	6.30
VI	ϕ Ac41	<1	10.46
	ϕ Ac42	<1	8.10
VII	ϕ Ac45	<1	9.21
	ϕ Ac46	<1	8.22
VIII	ϕ Ac57	<1	8.51
IX	ϕ Ac59	<1	6.50
X	ϕ AcM ₂	1,628	<1
	ϕ AcM ₃	1,616	<1
	ϕ AcM ₄	651	<1
	ϕ AcM ₅	616	<1
XI	ϕ AcS ₁	<1	<1
XII	Other C19 phages	<1	<1

^a Per minute.

Caulobacter strains. In addition, Schmidt's strain ϕ CFD-1, which is active against at least nine *Caulobacter* strains, was tested in the

present study and found to lyse or inhibit every *Asticcacaulis* strain used here except AC48.

Host range studies can also indicate relatedness between species and strains of the same genus. In this study, S-1 and S-3 behaved exactly the same, and C19 and AC2 showed nearly identical susceptibilities. It was then discovered that the original isolates S-3 and C19 of Pate and Ordal had been redesignated by other workers as S-1 and AC2, respectively (Schmidt, personal communication). Subculturing produced minimal changes, but otherwise the strains S-1 and S-3 are the same and C19 is the same as AC2.

Similar phage susceptibilities of S-3 and AC48 may indicate a close relationship between the two strains. The inability of *A. biprosthecum* phages to propagate on any other species and the inability of any other phages to propagate on C19 (although Schmidt's ϕ CFD-1 and *Asticcacaulis* phages, as well as 14 of our phages, will inhibit C19) indicate that *A. biprosthecum* is the most genetically isolated of the *Asticcacaulis* species studied here.

One of the original goals of this work was to find a transducing phage to use as a tool in studying the genetics of *Asticcacaulis*. Therefore, attempts were made to detect lysogeny in the available strains of *Asticcacaulis*. No temperate phages were found, and it has not yet been ascertained whether the phages isolated here are capable of transduction. Further work will therefore be required to obtain a viral system for gene transfer.

The phages isolated in this study are all morphologically similar to caulophages isolated elsewhere. Those DNA phages with heads that are hexagonal in outline, such as phage ϕ Ac31, are similar to Schmidt's DNA *Asticcacaulis* phages such as ϕ Ac20 (14) and to Driggers and Schmidt's temperate and defective DNA *Caulobacter* phages with the noncontractile tails (6). The DNA phages with elongated heads and long tails (ϕ Ac41, 42, and S₁) are similar to known, large DNA caulophages, with ϕ Ac41 and 42 most closely resembling Schmidt's

FIG. 10. Typical *Asticcacaulis* phage plaque morphologies. Magnification, $\times 0.5$. (A) ϕ Ac13 plated on *Asticcacaulis excentricus* AC48. (B) ϕ Ac31 plated on AC48. (C) ϕ Ac36 plated on AC48. (D) ϕ Ac37 plated on AC48. (E) ϕ Ac42 plated on AC48. (F) ϕ Ac45 plated on AC48. (G) ϕ Ac57 plated on AC48. (H) ϕ AcJ₁ plated on *A. biprosthhecum* C19. (I) ϕ AcS₂ plated on C19. (J) ϕ AcM₃ plated on C19.

phages ϕ Cb3, 6, and 13 (14) and ϕ AcS₁ most closely resembling Agabian-Keshishian and Shapiro's phage ϕ CbK (2). Phage ϕ AcM₂ is also similar to the other elongated caulophages, but it has a shorter head. No small spherical phages, such as Schmidt's ribonucleic acid phages ϕ Cb8r, 12r, or 23r, were seen (14).

ACKNOWLEDGMENTS

We are very grateful to Jean M. Schmidt of Arizona State University for providing us with 37 caulobacter strains and 3 caulophages.

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin at Madison, and by Public Health Service grant 1-RO1-GM 19859 from the National Institute of General Medical Sciences.

REPRINT REQUESTS

Address reprint requests to: Dr. J. L. Pate, Department of Bacteriology, University of Wisconsin, Madison, Wis. 53706.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Agabian-Keshishian, N., and L. Shapiro. 1970. Stalked bacteria: properties of deoxyribonucleic acid bacteriophage ϕ CbK. *J. Virol.* 5:795-800.
3. Agabian-Keshishian, N., and L. Shapiro. 1971. Bacterial differentiation and phage infection. *Virology* 44:46-53.
4. Bradley, D. E. 1962. A study of the negative staining process. *J. Gen. Microbiol.* 29:503-516.
5. Bradley, D. E. 1966. The fluorescent staining of bacteriophage nucleic acids. *J. Gen. Microbiol.* 44:383-391.
6. Driggers, L. J., and J. M. Schmidt. 1970. Induction of defective and temperate bacteriophages in *Caulobacter*. *J. Gen. Virol.* 6:421-427.
7. Henrici, A. T., and D. E. Johnson. 1935. Studies of freshwater bacteria. II. Stalked bacteria, a new order of schizomycetes. *J. Bacteriol.* 30:61-93.
8. Jollick, J. D. 1972. Differential phage sensitivity of cell types in *Caulobacter*. *J. Gen. Virol.* 16:405-407.
9. Jollick, J. D., and E. M. Schervish. 1972. Genetic recombination in *Caulobacter*. *J. Gen. Microbiol.* 73:403-407.
10. Kurn, N., S. Ammer, and L. Shapiro. 1974. A pleiotropic mutation affecting expression of polar development events in *Caulobacter crescentus*. *Proc. Natl. Acad. Sci. U.S.A.* 71:3157-3161.
11. Leffler, S., M. Hierowski, J. S. Poindexter, and W. Szer. 1971. Large scale isolation of the caulobacter bacteriophage ϕ Cb5 and its RNA genome. *FEBS Lett.* 19:112-114.
12. Pate, J. L., J. S. Porter, and T. L. Jordan. 1973. *Asticcacaulis biprothecum*: life cycle, morphology and cultural characteristics. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 39:569-583.
13. Poindexter, J. S. 1964. Biological properties and classification of the *Caulobacter* group. *Bacteriol. Rev.* 28:231-295.
14. Schmidt, J. M., and R. Y. Stanier. 1965. Isolation and characterization of bacteriophages active against stalked bacteria. *J. Gen. Microbiol.* 39:95-107.
15. Shapiro, L., and N. Agabian-Keshishian. 1970. A specific assay for differentiation in the stalked bacterium *Caulobacter crescentus*. *Proc. Natl. Acad. Sci. U.S.A.* 67:200-203.
16. Shapiro, L., N. Agabian-Keshishian, and I. Bendis. 1971. Bacterial differentiation. *Science* 173:884-892.
17. Shapiro, L., N. Agabian-Keshishian, A. Hirsch, and O. M. Rosen. 1972. Effect of dibutyl cyclic 3':5'-AMP on growth and differentiation in *Caulobacter crescentus*. *Proc. Natl. Acad. Sci. U.S.A.* 69:1225-1229.
18. Siddiqui, A., E. Hart, U. Mandagere, and I. D. Goldberg. 1974. Rapid plate method for the isolation of lysogenic bacteriophages. *Appl. Microbiol.* 27:278-280.