JOURNAL OF VIROLOGY, Nov. 1973, p. 1031-1033

Vol. 12, No. 5
Printed in U.S.A.

Isolation and Characterization of a Bacteriophage of *Arthrobacter globiformis*

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Received for publication 30 July 1973

A bacteriophage which reproduces on *Arthrobacter globiformis* ATCC 8010 was isolated from soil. This bacteriophage, designated φAG8010, propagates either in soft agar or broth cultures of the host. Because of a slow adsorption rate, neither the latent period nor burst size was determined. The mature virion belongs to Bradley's group B and exhibits a hexagonal head measuring 69 nm (length) by 60 nm (width) attached to a sheathless tail 120 nm long. The buoyant density of the mature virion is 1.534 g/cm^3. The mature virion contains double-stranded DNA with a buoyant density of 1.722 g/cm^3 (equivalent to 63.3% G + C). Of 14 strains (representing 13 species) of *Arthrobacter* examined, including *A. globiformis* ATCC 4336, only *A. globiformis* ATCC 8010 supported replication of φAG8010.

Only a limited number of reports of bacteriophages active on *Arthrobacter* spp. have been made. Conn et al. (5) reported the isolation of several bacteriophages specific for the *Bacterium globiforme* group and their use in the taxonomy of these soil bacteria. Gillespie (9) isolated and partially characterized a bacteriophage for *Arthrobacter globiformis* strain 616. Schippers-Lammertse et al. (14) described a new species, *Arthrobacter polychromogenes*, and reported the isolation of a bacteriophage active on this species. Daems (7) reported some of the fine-structure features of the phage active on *A. polychromogenes*.

This report describes the isolation and partial characterization of a bacteriophage from soil which is active on *Arthrobacter globiformis* ATCC 8010.

MATERIALS AND METHODS

Cultures of *Arthrobacter globiformis* ATCC 8010 and 4336, *A. histidinolovorans* ATCC 11442, *A. citreus* ATCC 11624, *A. simplex* ATCC 13260, *A. pascens* ATCC 13346, *A. atrocyaneus* ATCC 13752, *A. ramosus* ATCC 13727, *A. oxydans* ATCC 14358, *A. polychromogenes* ATCC 15216, *A. albidus* ATCC 15243, *A. variabilis* ATCC 15753, *A. crystallopoietes* ATCC 15481, and unidentified *Arthrobacter* sp. I-16 were employed. All cultures except *Arthrobacter* sp. I-16, a soil isolate, were obtained from the American Type Culture Collection, Rockville, Md.

Soil bacteriophage were isolated by the method of Adams (1). Logarithmically growing cultures of *A. globiformis* 8010 in nutrient broth (Difco) plus 0.2% yeast extract (Difco) received about 1 g of soil; incubation with gentle shaking was continued at 30 C for an additional 48 h. The cultures were then clarified by centrifugation, and the supernatant fluids were passed through an 02 Selas filter (0.4 μm average pore diameter, Selas Flotronics, Spring House, Pa.). Samples of the filtrate were assayed for plaque-forming units by the soft-agar overlay technique (1) with nutrient agar and soft agar containing 0.2% yeast extract. In later experiments it was found that trypticase soy broth (BBL) + 0.2% yeast extract (TSYE broth) and TSYE agar and soft agar were preferable for plaque assays, broth propagation, and other manipulations involving φAG8010.

Phage DNA was prepared by the method of Freifelder (8), followed by dialysis against BPES buffer (6). DNA was assayed by the diphenylamine reaction (3). Analytical ultracentrifugation determinations of buoyant densities of phage particles and DNA (4, 11) were performed using either *Escherichia coli* B DNA (1.710 g/cm^3) or staphylococcal bacteriophage 81 (1.476 g/cm^3) as references at 44,000 rpm.

For electron microscopy, phage particles in suspension medium (15) were negatively stained with 2% sodium phosphotungstate (pH 7.0) and were observed with an AEI Corinth 275 electron microscope. Average dimensions of the bacteriophage were determined using the lattice structure of crystalline bovine catalase (Calbiochem, La Jolla, Calif.) as standard (G. J. Berg, personal communication).

RESULTS

A sample of soil originating in northwestern Arkansas yielded plaques on *A. globiformis* 8010. No plaques were obtained on *A. atrocyaneus* 13752 and *Arthrobacter* sp. I-16

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when these strains were used in the enrichment procedure with this same soil sample. A single plaque on the lawn of *A. globiformis* 8010 was stabbed and used to prepare a high-titered lysate by serial passage on strain 8010 by the agar overlay technique. This phage was designated φAG8010.

Attempts to prepare lysates of φAG8010 in broth cultures of strain 8010 were only occasionally successful when nutrient broth plus 0.2% yeast extract was used. When TSYE broth was used as described below, however, lysates with reasonably high titers were routinely obtained. Strain 8010 was inoculated into 100 ml of TSYE broth in a 300-ml nephelometer flask, which was then shaken at 30 C until logarithmic growth ensued, as judged from the absorbancy of the culture. At the time of infection, the absorbancy at 545 nm was between 0.25 and 0.3. The culture was then infected at a multiplicity of infection between 3 and 5, as judged from total cell counts obtained with a Petroff-Hauser counting chamber and phase optics. Incubation with shaking at 30 C was continued. After an incubation period of 8 h, titers of free phage between 10^9 and 10^{10} PFU/ml were invariably obtained despite the fact that complete lysis (clearing of the culture) often required 18 to 24 h of incubation after infection. Residual cells and debris were removed by centrifugation, and the supernatant fluid was passed through a membrane filter (Millipore type HAWP047, Millipore Corp., Bedford, Mass.) and stored at 4 C.

All of the strains of *Arthrobacter* listed in the Materials and Methods were tested for their ability to support the growth of φAG8010. Soft agar overlays were prepared using each *Arthrobacter* sp. and 10^9, 10^5, and 10^2 PFU of φAG8010. The plates were incubated at 30 C and examined daily for 4 days for evidence of plaques. Only *A. globiformis* 8010 exhibited plaques. In our hands, plaques of φAG8010 are discernible in TSYE soft agar lawns of strain 8010 after 24 h at 30 C; however, accurate scoring of these plaques, observations of plaque morphology, etc., are best performed after 36 to 48 h of incubation.

Electron microscope observations of φAG8010 (Fig. 1) reveal a particle which best fits Bradley's group B (2). The phage possesses a hexagonal head measuring 60 nm wide and 69 nm long, attached to an unsheathed tail 120 nm long and about 10 nm wide; the distal 15 to 20 nm of the tail appear slightly thicker, suggesting an attachment organelle. No fibers have been observed associated with the tail.

When centrifuged to equilibrium in CsCl, φAG8010 exhibits a buoyant density of 1.534 g/cm^3. The DNA extracted from φAG8010 has a density of 1.722 g/cm^3; this density corresponds to a guanine plus cytosine (G + C) content of 63.3%. The thermal transition curve of φAG8010 DNA exhibits an increase in absorbancy characteristic of double-stranded DNA, with a midpoint (T_m) which is characteristic of DNA containing 63.8% G + C.

Attempts to obtain a one-step growth curve of φAG8010 on strain 8010 have thus far been unsuccessful because of the slow adsorption kinetics. Despite varying the organic and inorganic environments, pH and temperature, only about 50% of the PFU adsorb in 5 min, and after 15 min as many as 25% of the original PFU remain unattached. Although arthrobacters characteristically undergo morphological changes during their growth cycle, no significant difference in adsorption kinetics could be demonstrated when cell populations predominantly in the rod or coccal form were incubated with φAG8010. The specificity of a phage for only the motile, nonstalked stage of *Caulobacter* has been reported (10), and other examples undoubtedly are known.

DISCUSSION

With the exception of an apparently narrow host range and slow adsorption kinetics, φAG8010 appears to be a perfectly conventional phage active on *A. globiformis* 8010. Except for an apparent difference in the terminus of the phage tail, φAG8010 is similar in size and morphology to the *A. polychromogenes* phage isolated by Schippers-Lammertse et al. (14) and studied by electron microscopy by Daems (7). Furthermore, the base composition of the phage

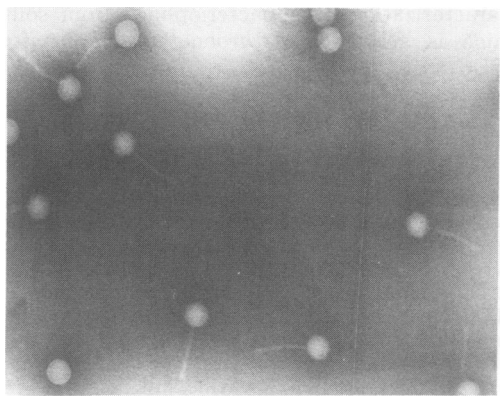

FIG. 1. *Electron micrograph of φAG8010 mature virions.* × *60,000.*

DNA is within the range reported previously for *A. globiformis* (12).

The slow adsorption kinetics of ϕAG8010 may reflect the inherent nature of the phage, or the failure to provide the most appropriate environment for adsorption. The extremely narrow host range, taken together with the slow adsorption kinetics, also suggests the possibility that ϕAG8010 is, in fact, a phage whose normal host is a microorganism other than *A. globiformis*. This possibility appears worthy of some further consideration when the origin of the phage from soil, and the variety of microorganisms in this environment, are taken into account.

Whatever the normal host of ϕAG8010 is, the existence of one or more bacteriophage active on the arthrobacters provides another means of studying the taxonomy of arthrobacters and closely related soil microorganisms. It is of greater practical significance, however, that the arthrobacters are present in the soil in significant numbers, and exhibit a variety of biosynthetic and degradative activities (13). The role of ϕAG8010 in facilitating "genetic communication" among members of the soil microflora, as well as offering a means for genetic analysis of these bacteria in the laboratory, remains to be determined.

ACKNOWLEDGMENTS

We thank M. E. Neville for performing the thermal denaturation analysis. Publication of this paper was supported in part by Public Health Service grant AI-06647 from the National Institute of Allergy and Infectious Diseases. J. A. Miller was a National Science Foundation Undergraduate Research Participant.

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