

BACTERIOPHAGES INFECTING
MICROMONOSPORA PURPUREA

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Although bacteriophages attacking actinomycetes have been studied for over 40 years^{1,2,3)}, the focus has been primarily on phages attacking streptomycetes and only one report⁴⁾ is available on an actinophage attacking *Micromonospora* species. We have undertaken a study of phages infecting various species of *Micromonospora* and wish to report our observations on two types of phages infecting *M. purpurea* ATCC 15835.

Soil samples from Florida and Wisconsin were used as source material for these phages. In one series of experiments water-extracts of the soils were sterilized by addition of CHCl_3 and the aqueous layer added to shaken flasks of the *M. purpurea* host. Tests were made on the cell-free liquid for phage using an agar-plating technique and more than half of the soil extracts examined were found to contain phage. In another experimental series cells of *M. pur-*

purea were added to soil samples and the samples incubated at 30°C for 9 days. After this incubation, the soil was eluted with medium and aliquots of the medium were tested for presence of phage by a spot test technique with *M. purpurea* ATCC 15835 as indicator strain.

Phage purification and assay were carried out by the conventional double-layer technique⁵⁾, and several different types of plaque-forming agents were detected. Phage ϕUW21 (isolated from the Florida soil) produces small and turbid plaques, while phage ϕUW51 (isolated from the Wisconsin soil) forms small and clear plaques.

Phage-containing lysates were prepared by adding the purified phage to 1 day old cells of *M. purpurea* growing in a starch-glucose-yeast extract-casein hydrolyzate- CaCl_2 medium in shaken flasks at 30°C. After two days' incubation the contents of the flasks were centrifuged at 8,000 rpm for 20 minutes and the phage lysates sterilized by filtration through a Millipore[®] filter (0.45 μ). The phage was pelleted by centrifuging at 36,000 rpm for 90 minutes in a Beckman L3-50 Ultracentrifuge. The pellets were resuspended in 0.1 M ammonium acetate and recentrifuged. The washed phage was then resuspended in 0.01 M ammonium acetate and

Fig. 1. Phage ϕUW21 ; $\times 161,000$ ($\times 38\%$)

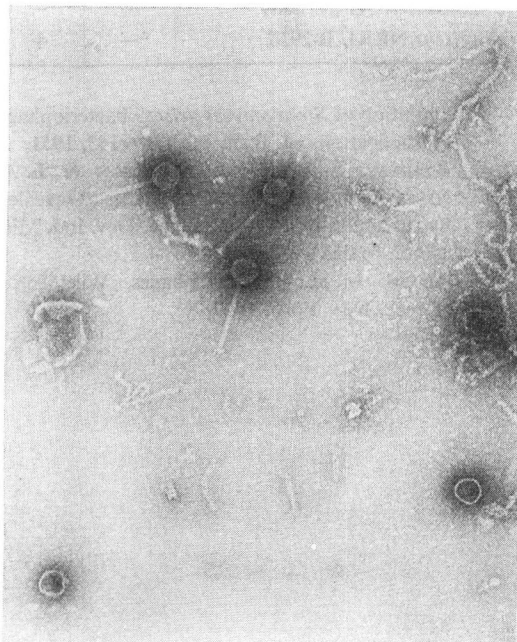
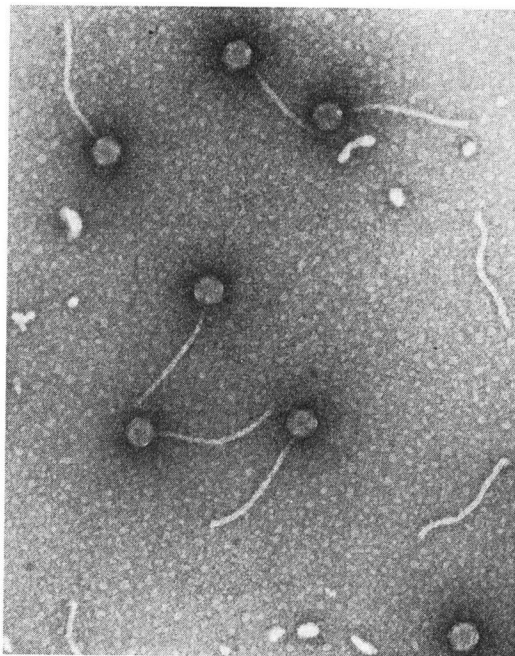


Fig. 2. Phage ϕUW51 ; $\times 161,000$ ($\times 38\%$)



studied for host range and morphology.

The pictures obtained with an electron microscope are shown in Figs. 1 and 2. Phage ϕ UW21 has short straight tails and hexagonal heads while phage ϕ UW51 has very long and curved tails and hexagonal heads. The approximate measurements for ϕ UW21 are 63 nm for the head width and 155 nm for the tail length, and the ϕ UW51 has 71.4 nm head width and 274.5 nm tail length.

The host range of ϕ UW21 and ϕ UW51 were examined by the cross-brush technique and some of the data collected are summarized in Table 1. Phage ϕ UW21 is a monovalent phage attacking only *M. purpurea* ATCC 15835 and phage ϕ UW51 is polyvalent attacking many strains of *Micromonospora*, one strain of *Streptomyces griseus* (of two tested) and also *Nocardia mediteranei* ATCC 13685. Additional information on the properties of these phages and others will be reported in the near future.

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Table 1. Host-range of phages

Host	Lysis observed	
	ϕ UW21	ϕ UW51
<i>Micromonospora purpurea</i> ATCC 15835	+	+
<i>M. echinospora</i> subsp. <i>echinospora</i> ATCC 15837	-	+
<i>M. echinospora</i> var. <i>pallida</i> ATCC 15838	-	+
<i>M. halophytica</i> subsp. <i>halophytica</i> ATCC 27596	-	+
<i>M. megalomicea</i> subsp. <i>nigra</i> ATCC 27598	-	+
<i>M. inyoensis</i> ATCC 27600	-	+
<i>M. coerulea</i> ATCC 21945	-	+
<i>M. chalcea</i> ATCC 12452	-	+
<i>M. carbonacea</i> ATCC 27114	-	+
<i>M. olivoasterospora</i> ATCC 21819	-	+
<i>Nocardia mediteranei</i> ATCC 13685	-	+
<i>N. aurantia</i> ATCC 12674	-	-
<i>N. calcarea</i> ATCC 19369	-	-
<i>Streptomyces griseus</i> ATCC 27001	-	-
<i>S. albus</i> NRRL B-2208	-	-
<i>S. venezuelae</i> NRRL B-2277	-	-
<i>S. viridochromogenes</i> NRRL B-1511	-	-
<i>S. flaveolus</i> NRRL B-2688	-	-
<i>S. loidensis</i> ATCC 11415	-	-
<i>S. kanamyceticus</i> ATCC 21486	-	-
<i>S. lavendulae</i> ATCC 8664	-	-
<i>S. griseus</i> NRRL B-2926	-	+

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