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Arthur Zachary

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## NOTES

### Isolation of Bacteriophages of the Marine Bacterium *Beneckea natriegens* from Coastal Salt Marshes<sup>1</sup>

ARTHUR ZACHARY

Virginia Institute of Marine Science Gloucester Point, Virginia 23062

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Bacteriophages of the marine bacterium *Beneckea natriegens* were isolated from coastal marshes where they were limited to brackish and marine waters. The phages were widely distributed and morphologically diverse in the marshes.

Numerous studies have established that bacteriophages are present in marine and estuarine waters (1, 3-16); however, few attempts have been made to estimate distribution, abundance, and the significance of bacterial viruses in marine ecosystems. Two more extensive studies have provided some indications of the abundance of bacteriophages, especially in coastal marine habitats. Ahrens (1), using two well-studied agrobacteria, showed that agrophages were widely distributed and abundant in the Baltic Sea. J. A. Baross (Ph.D. thesis, Univ. of Washington, Seattle, 1972) collected over 100 isolates of lytic and temperate bacteriophages active against marine vibrios from shellfish and intertidal shellfish growing areas in the Puget Sound area of Washington. This note reports the first isolation of bacteriophages of the marine bacterium *Beneckea natriegens* (ATCC no. 14048) and presents evidence of their wide distribution and morphological diversity in coastal marshes.

Mud samples (1 to 2 g) were collected in the intertidal zone, at the water's edge, by using a microcorer made from a disposable 10-ml syringe with its tip removed. The samples were treated with chloroform prior to enrichment with about  $10^8$  *B. natriegens* cells in 20 ml of nutrient broth made with estuarine water (14 to 17‰). After shaking at 150 rpm for 24 h at 27 C, the samples were again treated with chloroform. The lysates were centrifuged at low speed ( $4000 \times g$  for 20 min) to remove cell debris and tested for plaque-forming ability by spotting on lawns of *B. natriegens*. For electron microscopy, active lysates were passed through several enrichments and harvested as before. They were then centrifuged at high speed ( $30,000 \times g$ ) for 1 h. The pellets were then suspended in 2 ml of NaCl-MgCl<sub>2</sub>-tris(hydroxymethyl)-aminomethane buffer at pH 7.0, negatively stained with 1% uranyl acetate, and examined.

*Beneckea* phages were isolated from samples collected along the Atlantic coast, especially in the Chesapeake Bay, and also from one sample from the Gulf coast (Table 1). The presence of phages in many (in one case all) of the locations

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TABLE 1. Summary of field and laboratory observations

Location	Salinity (‰)	Temp (C)	No. of samples	Phage presence	Phage type present <sup>a</sup>
Gloucester, Mass.	31	24	2	+	1-5
Staten Island, N.Y.	24	37	2	+	1, 2, 4
Eastern Shore, Va.	30	25	4	+	1-4
Chesapeake Bay, Va.	8-18	24-27	50	+	1-4, 6
	1-8	24-27	50	±	1, 4, 6
	<0.5 <sup>b</sup>	23-26	30	-	
Sapelo Island, Ga.	30	27	1	+	1, 3, 4
Davis Bayou, Miss.	9	25	1	+	1

<sup>a</sup> Numbers refer to Fig. 1 to 6.

<sup>b</sup> Freshwater areas not subject to tidal influence.

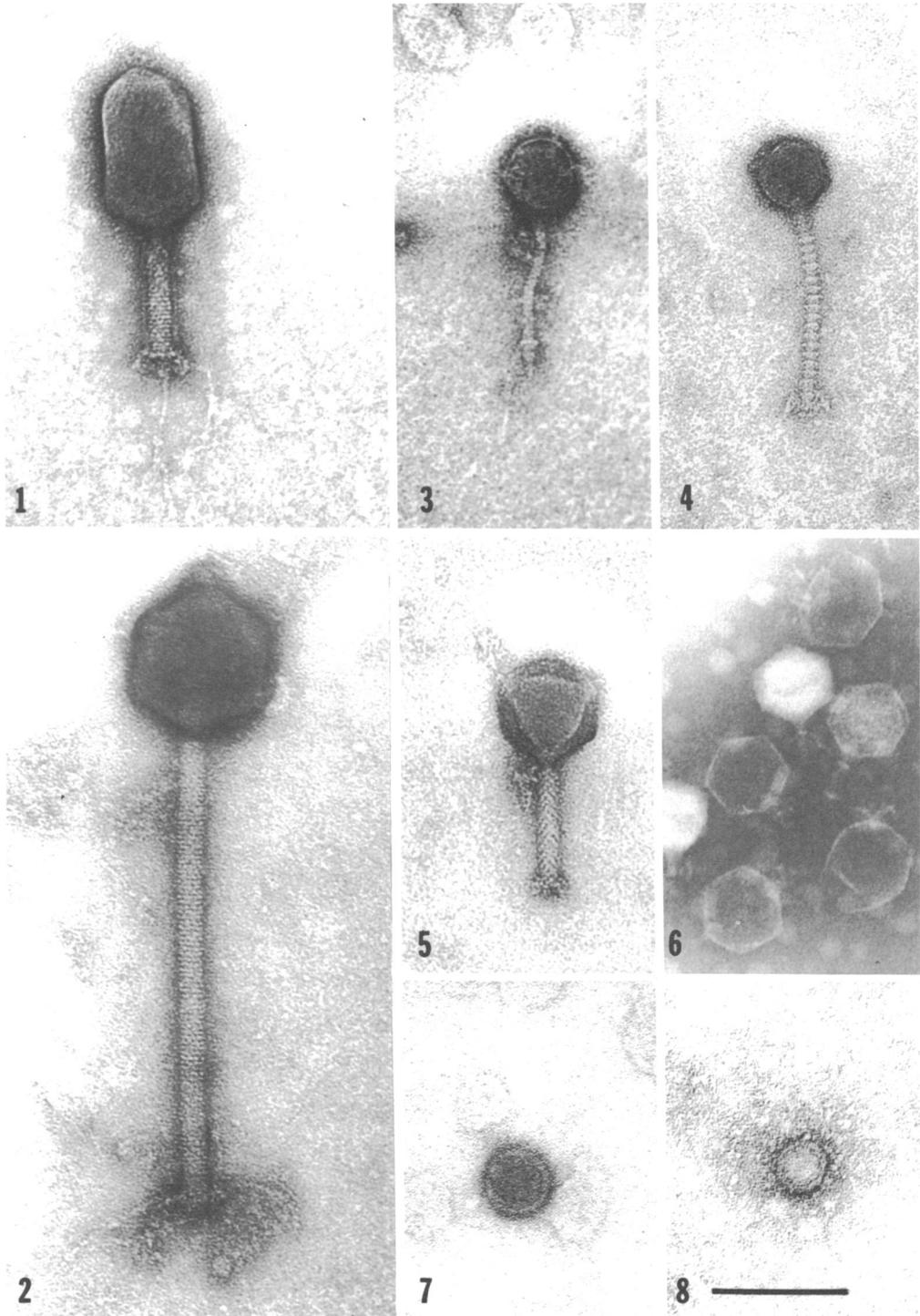


FIG. 1-8. *Benecke* phages from various samples negatively stained with uranyl acetate. Figures 1, 2, 4, and 5 are Bradley (2) group A phages. Figure 3 is a group B phage, Fig. 6 is a group C phage, and Fig. 7 and 8 are possibly group D or E phages. All photographs are at the same magnification; bar equals 100 nm.

reflects their wide distribution in the coastal marshes. The ease with which the phages were isolated from small samples, and their presence in all samples with salinity greater than 8‰, suggested that the phages were also abundant in these marshes. Those phages observed most often and present in high numbers in the lysates are shown in Fig. 1 to 6. The phages were highly diverse in that these samples included representatives of all the morphological categories of double-stranded deoxyribonucleic acid phages proposed by Bradley (2). In addition, numerous small virus-like particles were seen in most samples; however, they were not distinctive enough to allow positive identification or comparisons between samples (Fig. 7, 8). Only chloroform-resistant, lytic *Benecke* phages were isolated in this study. Other phages, such as chloroform-labile and temperate phages, may also occur, and the abundance and diversity of all *Benecke* phages is likely to be much greater than indicated in this study. Some of the *Benecke* phages resemble phages of non-marine bacteria and may have merely represented run-off into coastal waters. However, the reduced incidence of phage in low salinity areas and their absence in 30 samples from freshwater areas of Chesapeake Bay indicated that their distribution was salinity dependent (Table 1). Ahrens (1) showed a salinity-dependent distribution for agrophages in Kiel Bay. Thus, *Benecke* and *Agrobacterium* phages, and perhaps phages of other marine bacteria, are confined to brackish and marine waters.

The presence of a diverse group of phages is not unique to *B. natriegens* but represents a common phenomenon among bacteria. The numerous reports of other marine phage-host systems suggest the existence of a large marine bacteriophage population, especially in nutrient-rich habitats, such as marshes. In light of current trends toward pollution and eutrophication of coastal marine waters, the presence of bacteriophages, which by their lytic and transducing activity modify marine bacterial popula-

tions, becomes increasingly important. Bacteriophages are a significant component of the marine microbial biota and should therefore be included in studies of microbial processes occurring in marine ecosystems.

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