JOURNAL OF VIROLOGY, Jan. 1970, p. 97-98
Vol. 5, No. 1

NOTES

Isolation of a Bacteriophage for *Bdellovibrio bacteriovorus*

TADAYO HASHIMOTO, D. L. DIEDRICH, AND S. F. CONTI

Departments of Microbiology and Botany, T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506

Received for publication 6 November 1969

A phage infective for *Bdellovibrio bacteriovorus* was isolated. Electron microscopy revealed that it is tail-less, has a hexagonal appearance and two distinct capsomere layers, and is 60 to 70 nm in size. The nucleic acid appears to be single-stranded deoxyribonucleic acid. This is the first report of the isolation of a phage infective for *B. bacteriovorus*.

Bdellovibrio bacteriovorus is a gram-negative, motile bacterium which is parasitic on a variety of gram-negative (3, 6) and some gram-positive (3) bacterial hosts. Although the organism usually infects and multiplies only within living bacteria, host-independent mutants which are capable of growing in an artificial medium have been reported (5, 6; R. J. Seidler and M. P. Starr, Bacteriol. Proc., p. 23, 1968). We have recently reported the isolation of a mutant (strain UK_{i2}), derived from a parasitic strain of *B. bacteriovorus*, which can grow either parasitically with *Escherichia coli* as the host or saprophytically in an artificial medium (D. L. Diedrich et al., Bacteriol. Proc., p. 53, 1969).

During the course of further characterization of this mutant, we isolated a phage for *B. bacteriovorus* strain UK_{i2} which possesses some unusual morphological characteristics.

The bacteriophage was isolated from a sample of local sewage, with *B. bacteriovorus* UK_{i2} as the host, by conventional isolation techniques (1). The phages were propagated on host cells in YP broth (7) and were concentrated by means of differential centrifugation. Negatively stained preparations were made on Formvar-coated grids; 0.5% uranyl acetate was used for staining. The host cells infected with phage were fixed with glutaraldehyde, postfixed in osmium tetroxide, dehydrated, embedded in Epon, and examined by using previously described procedures (4).

Electron micrographs of the negatively stained phages (Fig. 1) revealed that this phage is devoid of a tail and that the capsid appears to be composed of two distinct coats; the capsomeres are particularly evident in the inner coat. There are some indications (arrows in Fig. 1) that an additional layer surrounds the inner coat. The phage is about 60 to 70 nm in size and has a more or less regular hexagonal outline, which is also evident in thin sections of host cells infected with this phage (Fig. 2).

Our preliminary tests for identification of the nucleic acid type by using acridine orange staining combined with molybdic acid treatment (2) suggest that this phage contains single-stranded deoxyribonucleic acid.

Further characterization of this *B. bacteriovorous* phage and the susceptibility of other *Bdellovibrio* strains to it are currently being studied. We have designated this phage as HDC-1.

This investigation was supported by grant GB-5336 from the National Science Foundation.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Bradley, D. E. 1966. The fluorescent staining of bacteriophage nucleic acids. J. Gen. Microbiol. 44:383-391.
3. Burger, A., G. Drews, and R. Ladwig. 1968. Wirtskries und Infektionscyclus eines neues isolierten *Bdellovibrio bacteriovorus* Stammes. Arch. Mikrobiol. 61:261-279.
4. Burnham, J. C., T. Hashimoto, and S. F. Conti. 1968. Electron microscopic observations on the penetration of *Bdellovibrio bacteriovorus* into gram-negative bacterial hosts. J. Bacteriol. 96:1366-1381.
5. Shilo, M., and B. Bruff. 1965. Lysis of gram-negative bacteria by host-independent ectoparasitic *Bdellovibrio bacteriovorus* isolates. J. Gen. Microbiol. 40:317-328.
6. Stolp, H. 1963. *Bdellovibrio bacteriovorus* —ein räuberischer Bakterienparasit. Naturwissenschaften 55:57-63.
7. Stolp, H., and M. P. Starr. 1963. *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek J. Microbiol. Serol. 29:217-248.

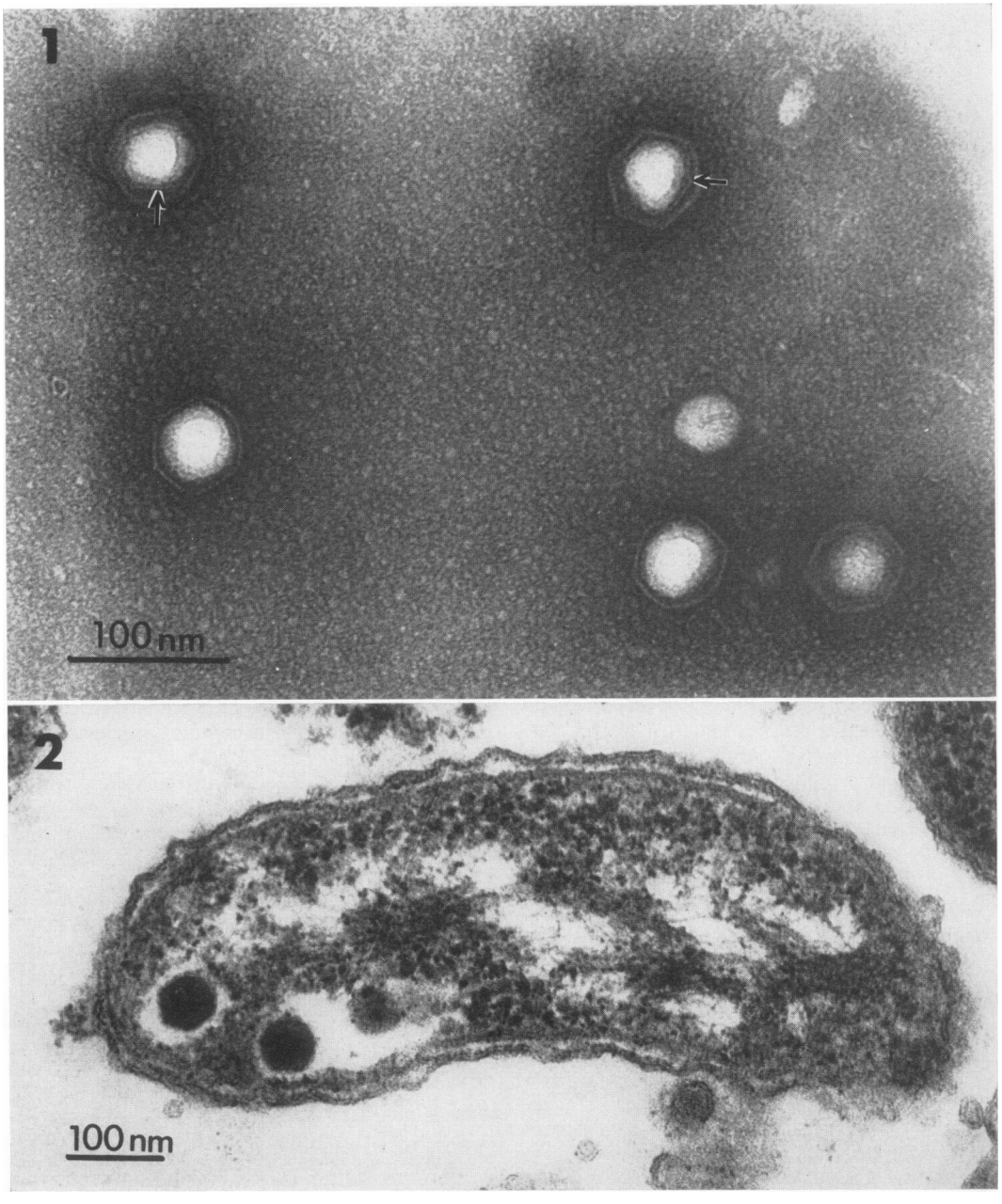

FIG. 1. *Negatively stained (0.5% uranyl acetate) bacteriophages for a facultatively parasitic strain (UK_{i2}) of Bdellovibrio bacteriovorus.*

FIG. 2. *Bdellovibrio bacteriovorus UK_{i2} 45 min after infection with the phage, illustrating the hexogonal appearance of intracellular phage particles.*