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NOTES

Morphology of Bacteriophage-Like Particles from *Fusobacterium symbiosum*

M. A. FOGLESONG AND A. J. MARKOVETZ
Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

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The morphology of bacteriophage-like particles from the strict anaerobe *Fusobacterium symbiosum* is described. Attempts to demonstrate plaque formation on the host strain of *F. symbiosum* and other related species were unsuccessful.

Comparatively few bacteriophages have been isolated from anaerobic bacteria. Those bacteriophages described have been mainly associated with four genera, *Lactobacillus* (4, 11), *Clostridium* (3, 5, 9), *Bacteroides* (7), and *Actinomyces* (8). This study is concerned with the description of a phage-like particle from the strict anaerobe, *Fusobacterium symbiosum*. Recently, Bradley (2) reported on the identification of a bacteriophage from *Sphaerophorus varius* (*Fusobacterium varium*) that differs from the particle described in this study.

F. symbiosum, isolated from the hind gut of a cockroach (*Blaberus* sp.), was grown on a peptone-yeast extract-glucose (PYG) prereduced medium utilizing the roll-tube technique and an anaerobic chamber. The culture was maintained on peptone-yeast extract-glucose agar plates and subcultured from a single colony every 3 days. The organism was grown at either 25 or 37 C in 5 to 10 ml of culture medium. Particles were prepared for electron microscope examination by two methods. (i) Bacterial cultures were grown to an optical density of 0.28 and the cells were pelleted by centrifugation at 5,000 × *g* for 15 min. After centrifugation, the supernatant fluid was removed and the pellet was resuspended in 1 or 2 drops of 1% ammonium acetate in 0.1% sucrose, pH 7.6. A drop of the suspended cells was placed on a parlodion-coated 300-mesh copper grid. After 2 min, the drop was removed from the grid by adsorption to a piece of filter paper, and the grid was washed gently in 1% ammonium acetate in 0.1% sucrose, pH 7.6. The specimen was stained for 45 s with 2% phosphotungstic acid in 0.01% sucrose, pH 7.6, dried, and examined immediately under the electron microscope (JEOL 100 B). (ii) Cells were removed from the medium as above, and the supernatant fluid was centrifuged at 54,000 × *g* in a Beckman model L-3 ultracentrifuge. After centrifugation, the supernatant fluid was discarded, and the pellet was suspended in a small amount of 1% ammonium acetate in 0.1% sucrose, pH 7.6. Particles were stained as above, except that the phosphotungstic acid was left on the grid for 15 to 20 s.

Large numbers of phage-like particles were found adsorbed to the surface of *F. symbiosum* (Fig. 1). In addition, many particles were observed attached to cellular debris associated with ghost cells. Morphology of the phage-like particles was typical of Bradley's group A bacteriophages (1). Particles possessed a hexagonal head and long tail with a contractile sheath terminating with a base plate and tail fibers (Fig. 2-4). The head was approximately 53 nm in diameter, and the tail tube measured approximately 8.5 nm in width and 158 nm in length. The intact sheath measured approximately 20 nm in width and 160 nm in length (Fig. 2). The gap between the head and tail (Fig. 2) may represent a small attachment tube as described for *Desulfovibrio* phage (6), or the gap may merely represent early degradation of the sheath protein, which appears at a more degraded state in Fig. 3 and 4. No collar was observed. The varied position of some contracted sheaths that are frequently observed in particles (Fig. 3, 4) also has been observed in the *Desulfovibrio* phage (6), a defective phage of *Bacillus subtilis* (10), and killer particles from *B. licheniformis* (1). The phage-like particle in Fig. 5 possessed a naked tail tube of twice the

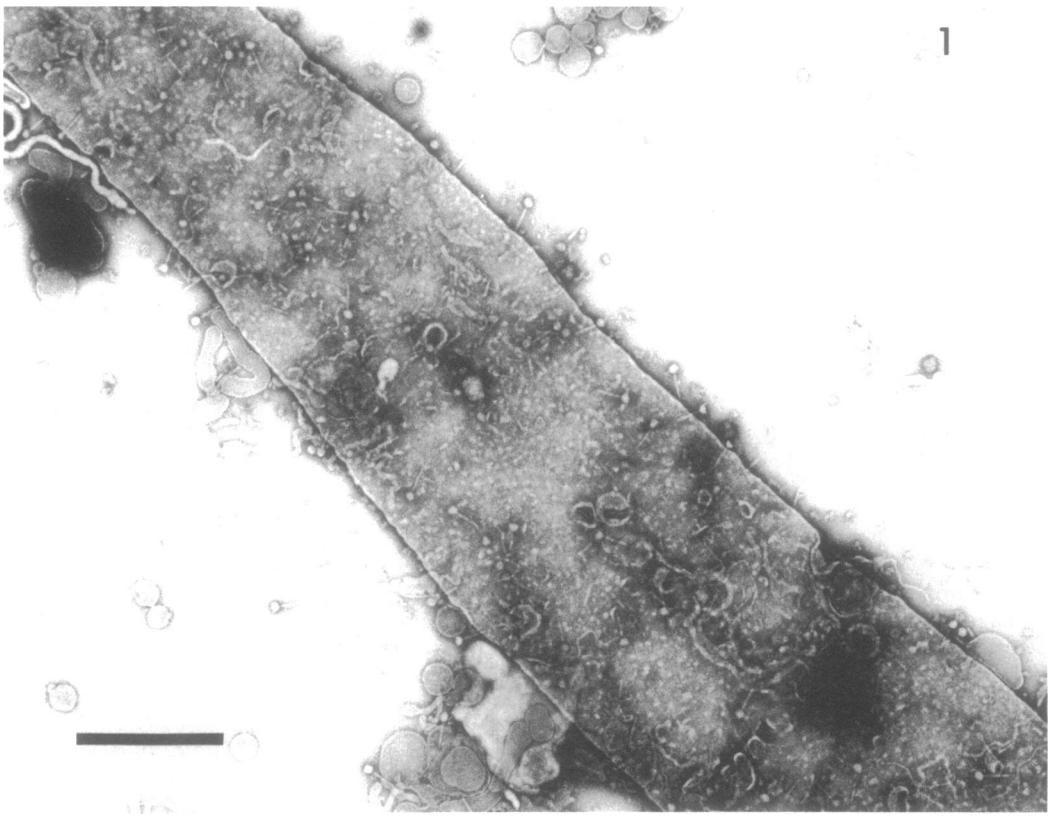

FIG. 1. *Electron micrograph of negatively stained F. symbiosum with numerous phage-like particles attached. Many particles can also be seen attached to debris in the area. Magnification bar = 1 μm.*

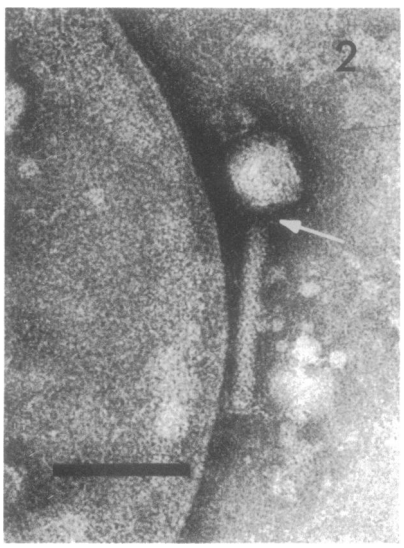

FIG. 2. *Electron micrograph of negatively stained F. symbiosum and phage-like particles. Arrow indicates the neck region between the head and extended sheath. Note also the base plate and tail fibers at the distal end of the tail. Magnification bar = 100 nm.*

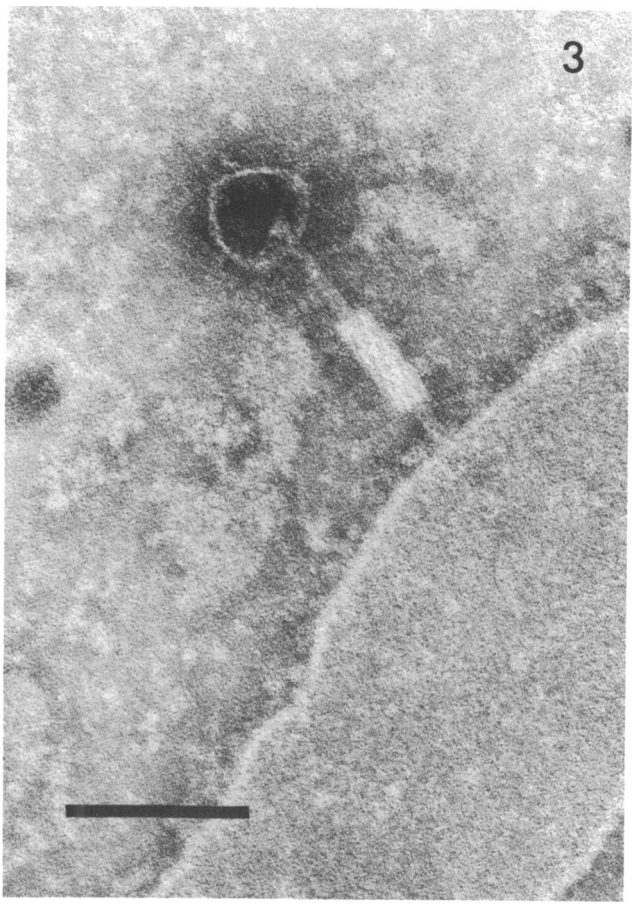

FIG. 3. *Electron micrograph of negatively stained particle attached to cellular debris. Note the unusual position of the contracted sheath on the tail tube. Magnification bar = 100 nm.*

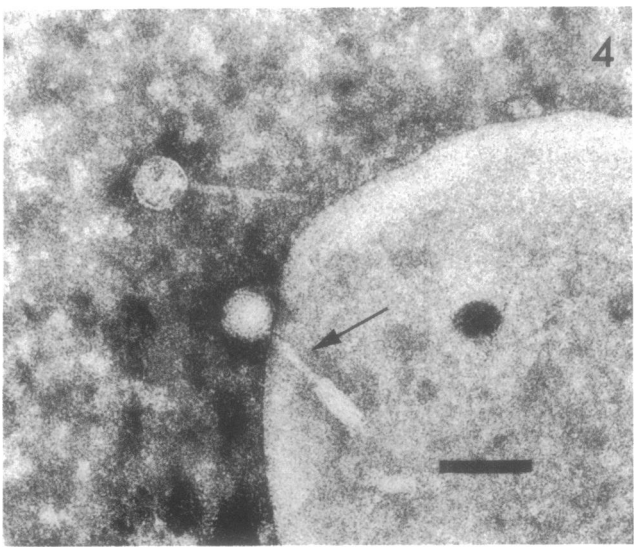

FIG. 4. *Electron micrograph of particles prepared by ultracentrifugation of supernatant fluid. Note the sheath position in the marked particle. Magnification bar = 100 nm.*

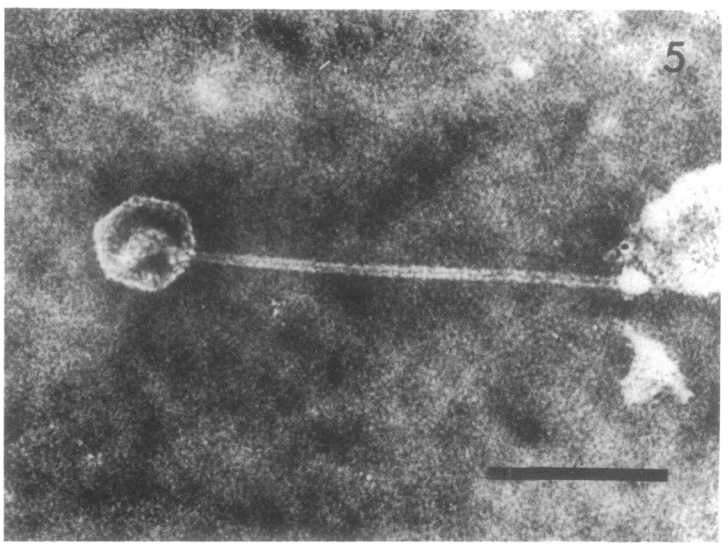

FIG. 5. *Electron micrograph of particle prepared by ultracentrifugation of supernatant fluid. The naked tail tube appears to be a double tube measuring approximately 246 nm in length. Note the easily distinguishable hexagonal head. Magnification bar = 100 nm.*

length (246 nm) of normal tail tubes. The phage head was empty and no sheath was associated with the tail tube. Particles of this nature appear to be the result of an abortive process.

Numerous attempts to demonstrate plaque formation on the host strain of *F. symbiosum* by conventional soft-agar overlay techniques were unsuccessful, as were similar attempts using three other host organisms, *S. varius* ATCC 8501 (*F. varium*), *Bacteroides funduliformis* ATCC 12290, and *Sphaerophorus intermedius* ATCC 23745 (*Bacteroides fragilis* subspecies *fragilis*). The unusual position of some of the contracted sheaths, as well as the existence of phage-like particles totally lacking sheaths, indicate that these particles are rather unstable.

This instability may account for the difficulty encountered in obtaining plaque formation on the host strain.

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