NOTES
Structure of the Flagellum of Bdellovibrio bacteriovorus

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*Bdellovibrio bacteriovorus* is a small bacterium which has the unique capacity to parasitize gram-negative bacteria. The unusually thick polar flagellum and the very active motility of *Bdellovibrio* may have a bearing on the contact necessary for successful parasitic interaction with the host bacteria.

In one of our earlier reports, the *Bdellovibrio* flagellum was described as about 55 μm in thickness (H. Stolp and M. P. Starr, Antonie van Leeuwenhoek J. Microbiol. Serol. 29:217, 1963), some 3 to 4 times thicker than typical bacterial flagella. These observations had been made with shadowed preparations, which did not allow precise measurements nor detection of any differentiation within the flagellum. In a more recent report, the absence of flagella on the parasite in thin-section preparations was noted, and it was suggested that the flagella had become detached during the dehydration or embedding procedures (M. P. Starr and N. L. Baigent, J. Bacteriol. 91:2006, 1966). In the present communication, we present our observations of negatively stained and thin-section preparations which clarify the size and some details of the structure of the *Bdellovibrio* flagellum.

Two parasitic strains of *Bdellovibrio* were investigated: A3.12 and 109 (H. Stolp and M. P. Starr, Antonie van Leeuwenhoek J. Microbiol. Serol. 29:217, 1963). One host-independent strain derived from A3.12 was isolated and used for the thin-section preparations (M. Shilo and B. Bruff, J. Gen. Microbiol. 40:317, 1965). The parasites were grown with their host bacteria (*Pseudomonas putida* A3.12 and *Escherichia coli* B, respectively) in the NB/10 broth used by Shilo and Bruff; the host-independent strain was grown in a medium consisting of 0.3% Difco yeast extract and 1% Difco peptone (PYE). All cultures were grown on a shaker at 28 C, harvested during the active phase of growth, and prepared for examination.

Cells of the host-independent strain were prefixed in glutaraldehyde (5% final concentration) for 1 hr. The glutaraldehyde was added dropwise from a 25% stock solution into an ice-cold culture suspended in PYE plus 0.1 m phosphate buffer (pH 6.8). Fixation and dehydration proceeded according to the method of Ryter and Kellenberger (Z. Naturforsch. 13b:597, 1958). The sapsrophytes were embedded in epoxy resins and sectioned with a diamond knife on a Porter-Blum ultramicrotome. Sections were mounted on 400-mesh copper grids and double-stained with lead citrate and uranyl acetate. The whole-mount preparations were prepared by adding formaldehyde dropwise into PYE broth (1% final formaldehyde concentration) and stained with either phosphotungstic acid (pH 7.2) or uranyl acetate. Other preparations were treated for 5 min with 6 m urea and stained with potassium iodide and ammonium molybdate (E. A. C. Follet and J. Gordon, J. Gen. Microbiol. 32:235, 1963). All specimens were examined in an RCA EMU-3 electron microscope.

The flagella of host-independent strain A3.12 were isolated in the following manner: Samples of an actively growing culture were spread on plates of PYE agar and incubated for 3 to 4 days at 30 C. The plates were flooded with 2 to 3 ml of distilled water, and the cells were removed with a curved glass rod. The cells were centrifuged at 10,000 x g for 30 min; the flagella remained in the supernatant fraction.

The gross features of the *Bdellovibrio* flagellum are shown in Fig. 1. The flagellum is of uniform width (about 28 μm) and typically possesses a decreasing amplitude and wavelength proceeding from the cell toward the flagellum tip; however, less ordered arrangements of flagella also are observed. The flagellum originates within the cell wall.

Negatively stained preparations of the flagella show that it consists of two portions: an inner core approximately 13 μm in thickness, and an outer sheath consisting of two dense regions separated by a less dense zone. The sheath thickness is approximately 7.5 μm (Fig. 2). This sheathed nature of the *Bdellovibrio* flagellum was recently
FIG. 1. *Parasitic Bdellovibrio bacteriovorus* strain 109, grown with its host in NB/10 broth. Fixed with 1% formaldehyde; stained with 0.5% uranyl acetate. × 43,000.

FIG. 2. Flagellum of parasitic *Bdellovibrio bacteriovorus* strain A3.12. Fixed with 1% formaldehyde; stained with 1% phosphotungstic acid, pH 7.2. The flagellum consists of the sheath (S) and the core (C). × 111,000.
FIG. 3. Flagella of parasitic Bdellovibrio bacteriovorus strain A3.12. After a 5-min urea treatment, the sheath swells (SS), breaks (B), and separates from the core (C). The sheath fragments into small uniform particles which are evident in the background (P). X 111,000.

FIG. 4-5. Thin-sections of host-independent Bdellovibrio bacteriovorus strain A3.12. Section through area of flagellar attachment, illustrating origin of sheath (S) from cell wall (CW). X 182,000 (Fig. 4); X 197,000 (Fig. 5).

1954

Figure 3 illustrates the effect of a 5-min exposure of isolated flagella to 6 M urea. The sheath swells up, breaks and separates from the core. Finally, the sheath fragments into smaller particles which are evident in the background. Figure 3 thus illustrates that the flagellum consists of two morphologically separable entities—the sheath and the core.

Thin sections permitted us to observe the origin of the sheath surrounding the flagellum. Rarely, a section was obtained through the area of attachment of the flagellum to the cell (Fig. 4 and 5). In such sections, it can be seen that the sheath surrounding the flagellum originates from the outermost component of the cell envelope, which we consider to be the cell wall. The width of the relatively thin cell wall of *Bdellovibrio* is essentially the same thickness as the sheath, about 7 to 7.5 μm.

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