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NOTES

Lysis of *Sphaerotilus natans* Swarm Cells by *Bdellovibrio bacteriovorus*

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Six strains of *Sphaerotilus natans* (smooth form) were lysed by five parasitic strains of *Bdellovibrio bacteriovorus*. The possible use of *Bdellovibrio* to control the proliferation of *S. natans* in the environment was hypothesized.

The sheathed bacteria of the genus *Sphaerotilus*, which proliferate abundantly in flowing polluted streams, cause serious impairment to the recreational and economic uses of the streams and gross deterioration of their aesthetic qualities (1). *Sphaerotilus* is also considered a nuisance organism in waste water treatment plants because it is usally associated with "bulking" of activated sludge (1, 2). Because attempts to control *Sphaerotilus* chemically have met with only limited success, other means of control must be devised to minimize the massive proliferative capability of this filamentous organism.

Since the discovery of the bacterial parasite *Bdellovibrio bacteriovorus* in 1962 (13), most research on this unusual microorganism has focused on attempts to elucidate its physiological, biochemical, and genetic properties (7, 10, 11). Several theories have been advanced to explain the important role *Bdellovibrio* must play in nature as a biological control agent (3-5, 8). To my knowledge, no one has attempted to utilize the bdellovibrios to control the growth of *Sphaerotilus natans*.

S. natans strains 5, 52, and 76 and *S. discophorus* strain 100D were kindly provided by W. L. van Veen, Agricultural University, Wageningen, The Netherlands. Additional strains of *S. natans* were obtained from the American Type Culture Collection (i.e., ATCC 13338 and 13339). Host-dependent (H-D) *Bdellovibrio* strains 109D, 110, and 114 were kindly provided by S. F. Conti, University of Kentucky. Host-dependent strains 6-5-S and 101 were obtained from J. Robinson, University of Western Ontario, Ontario, Canada, and M. P. Starr, University of California, Davis, respectively.

Advantage was taken of the fact that *Sphaerotilus* dissociates when cultivated in the laboratory from its characteristically filamentous colonial form (rough or R type) to a smooth or S type composed primarily of free-living, single cells (12). Swarm cells were grown overnight on a shaker at 30 C in CGY medium (2) containing Casitone (Difco), 0.5%; glycerol, 1.0%; and yeast autolysate, 0.1% (pH 7.2). *Bdellovibrio* cells were grown at 30 C on *S. natans* ATCC 13339 in either dilute nutrient broth (DNB) supplemented with Ca^{2+} and Mg^{2+} (6) or distilled water containing 0.001 M tris(hydroxymethyl)aminomethane (pH 7.5), 0.002 M $CaCl_2$, and 0.003 M $MgSO_4 \cdot 7H_2O$. Plaque counts were determined on lawns of *S. natans* ATCC 13339 by the double layer technique (0.6% DNB top agar, 1.0% DNB bottom agar).

Specimens were prepared for electron microscopy by fixing in 1.0% formaldehyde and staining directly on the grid with 1.0% phosphotungstic acid (pH 7.0). Specimens were examined in a JEM 100 B electron microscope (JEOL, Ltd., Tokyo) operating at 60 kV with a 60-μm objective aperture.

Two-membered cultures were established by harvesting and washing overnight cultures of *Sphaerotilus* swarm cells, resuspending the cells in 25 ml of DNB or buffered salts solution, inoculating with 1.0-ml quantities of *Bdellovibrio* lysates, and incubating at 30 C on a shaker. Uninoculated *Sphaerotilus* swarm cells were used as controls. Lysis was measured qualitatively by phase contrast microscopy.

All six strains of *Sphaerotilus* were lysed in 15 to 18 h by all *Bdellovibrio* strains tested.

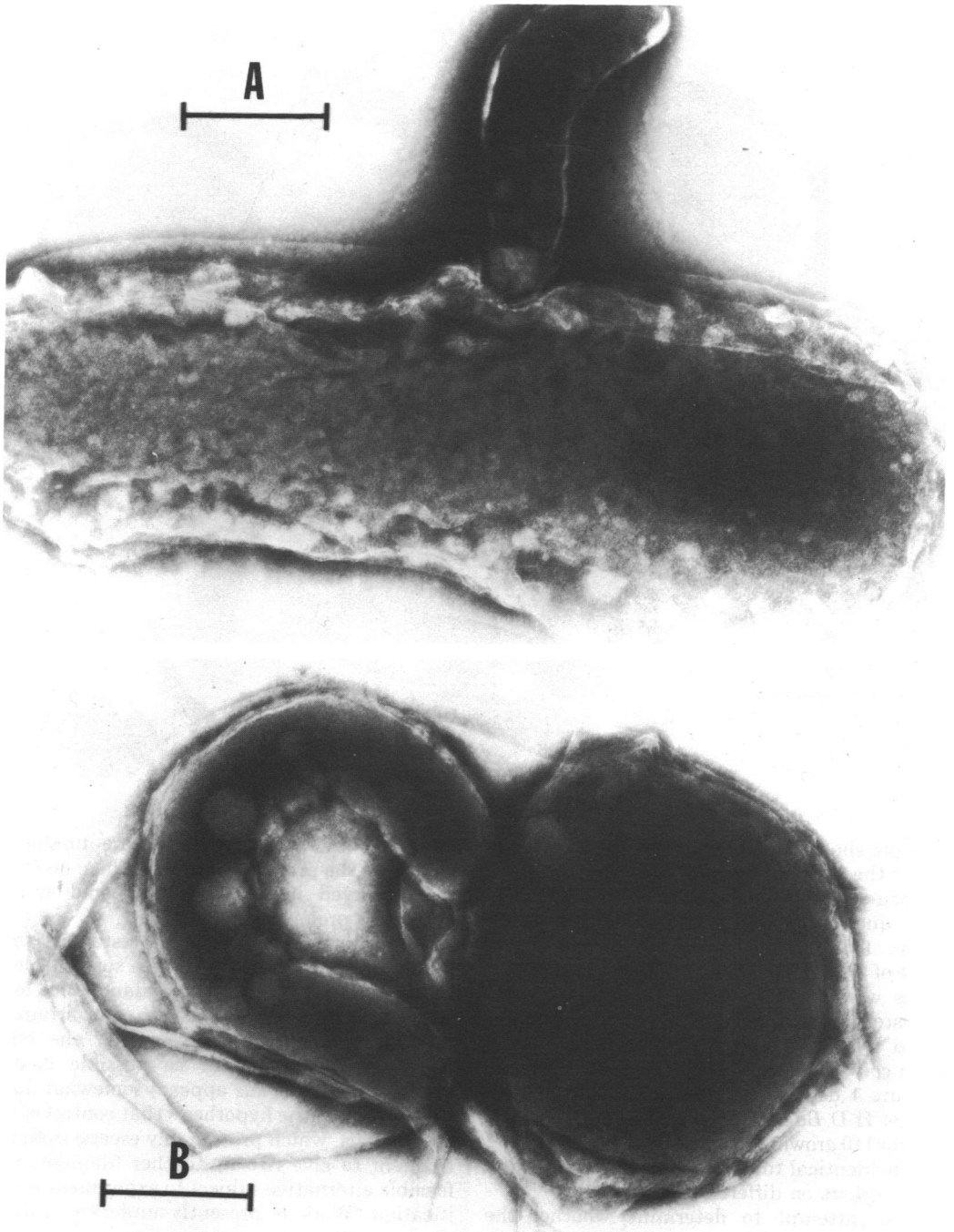

FIG. 1. *Two stages in the life cycle of B. bacteriovorus growing on S. natans 13339 in liquid culture. (A) H-D Bdellovibrio 6-5-S attached to a swarm cell of S. natans; (B) early elongation phase of H-D Bdellovibrio 6-5-S within the periplasmic space of S. natans 13339; (C) late elongation phase of H-D Bdellovibrio 110 within the periplasmic space of S. natans 13339. Bars in all figures represent 0.5 μm.*

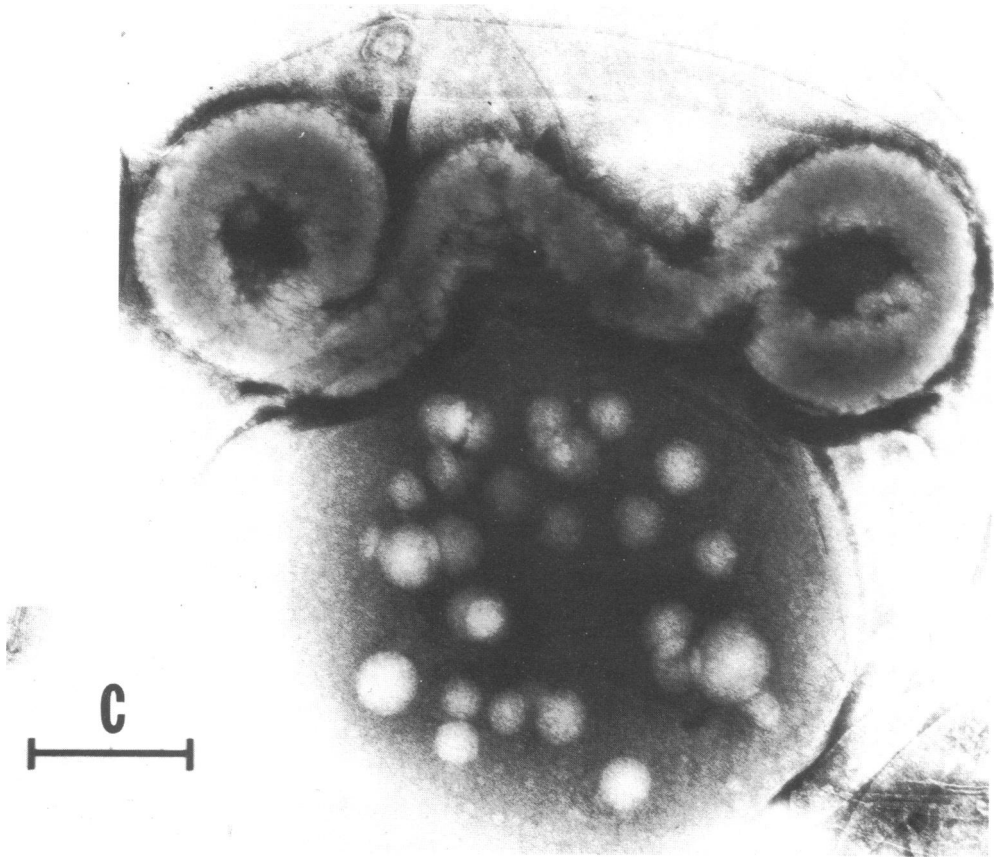

FIG. 1C

Controls showed no signs of autolysis or stress during the incubation time.

When growth of *B. bacteriovorus* was monitored quantitatively in liquid cultures of *S. natans* 13339 (i.e., by plaque formation on lawns of *S. natans* 13339), the ensuing growth curves were very similar to the characteristic multistep growth pattern of *Bdellovibrio* reported by Varon and Shilo (14) with *Escherichia coli* as host.

Figure 1 depicts negatively stained preparations of H-D *Bdellovibrio* 6-5-S and H-D *Bdellovibrio* 110 growing on *S. natans* 13339. The life cycle is identical to that previously reported by other workers on different hosts (9, 11).

In an attempt to determine whether the filamentous form of *S. natans* was susceptible to the lytic action of *B. bacteriovorus*, an overnight culture of *S. natans* strain 76, grown in Stokes medium (12) in place of CGY, was prepared as above and inoculated with 0.25 ml of a washed suspension of a fresh *Bdellovibrio* lysate (strain 110). Attachment was readily apparent, but the parasites were unable to penetrate the protective sheath. Thus, no effect was observed in this two-membered system even after prolonged incubation.

All strains of *Sphaerotilus* tested in their free-living, single-cell state were susceptible to lysis by *B. bacteriovorus*. The filamentous form was impenetrable, at least to one strain of *Bdellovibrio*. The speculation that the bdellovibrios could be used as possible control agents of *Sphaerotilus* appears somewhat dubious. However, the hypothesis that control of the swarm cells, which presumably escape from the filament to give rise to another filament, is a feasible alternative subject to experimental verification. Work is presently underway to test this hypothesis.

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