APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1990, p. 357–361
0099-2240/90/020357-05$02.00/0

Vol. 56, No. 2

Occurrence of a *Thiothrix* sp. Attached to Mayfly Larvae and Presence of Parasitic Bacteria in the *Thiothrix* sp.

JOHN M. LARKIN,[1]* MARGARET C. HENK,[1] AND SHERIL D. BURTON[2]

Department of Microbiology, Louisiana State University, Baton Rouge, Louisiana 70803,[1] and Department of Microbiology, Brigham Young University, Provo, Utah 84602[2]

Received 26 June 1989/Accepted 28 October 1989

Larvae of the mayfly (*Drunella grandis* [Eaton]) from Diamond Fork Creek, Utah, were covered with a heavy growth of the sulfide-oxidizing bacterium *Thiothrix*. The bacterium did not seem to harm the mayfly, but the *Thiothrix* trichomes were parasitized by three morphologically distinct bacteria, two of which were cytoplasmic and one of which was probably periplasmic. At least two of the parasites destroyed the cytoplasmic contents of the *Thiothrix* sp., thus killing the host cell. Attempts to obtain the parasites in pure culture were unsuccessful.

Several types of predation of bacteria upon other bacteria have been described. Some gliding organisms, such as certain myxobacteria (7) and *Herpetosiphon* spp. (8), glide over and around the prey while excreting enzymes that degrade the host. Others, such as those of the genera "*Ensifer*" (3), "*Cupriavidus*" (6), and "*Vampirococcus*" (4), adhere to the prey and presumably secrete enzymes which break down the host. "*Vampirovibrio*" organisms prey upon certain algae but do not penetrate the algal cell (2).

Only two bacteria that can penetrate the host bacterial cell wall have been described. *Bdellovibrio* spp. have been extensively studied (1); they penetrate the cell wall of the host and multiply within the periplasm. The bacterium "*Daptobacter*" is the only one that has been described as being able to penetrate the cytoplasm of the host and multiply there (4), but the evidence for this is as yet incomplete.

During a study of the relationship between a mayfly larva and the sulfide-oxidizing bacterium *Thiothrix* which was attached to the larval exoskeleton, three morphologically distinct internal parasites of the *Thiothrix* sp. were found. Two of them appear to be cytoplasmic, and the other may be cytoplasmic but more likely is periplasmic. The discovery of these organisms may provide additional evidence relating to the origin of organelles in eucaryotic organisms.

MATERIALS AND METHODS

Sample collection. Larvae of the mayfly *Drunella grandis* (Eaton), which were covered with a growth of *Thiothrix* organisms, were found on the bottom of rocks in Diamond Fork Creek, Utah, at a location where the effluent from a sulfur spring joined the fresh water of the creek. Larvae were transported to the laboratory for attempted isolation of the *Thiothrix* sp., and other larvae were fixed at the site for electron microscopic examination.

Electron microscopy. Specimens for transmission electron microscopy were fixed for 30 to 60 min with 2% glutaraldehyde plus 0.0125% ruthenium red in 0.1 M sodium cacodylate buffer, pH 7.0. They were rinsed for 3 to 8 days in 0.1 M buffer containing 0.0125% ruthenium red, postfixed in 2% aqueous osmium tetroxide for 1 h, rinsed in water, en bloc stained in 0.5% aqueous uranium acetate for 1 h in darkness, dehydrated in ethanol, and infiltrated and embedded in Epon araldite. Blocks were sectioned on an MT 5000 Sorvall ultramicrotome and viewed with a JEOL 100-CX microscope.

Samples for scanning electron microscopy were prepared identically to transmission electron microscopy specimens through ethanol dehydration, except that ruthenium red was omitted. After dehydration, specimens were critical-point dried, mounted, sputter coated with gold-palladium on a Hummer sputter coater, and viewed with a Hitachi S-5000 or JEOL 100-CX microscope.

RESULTS

Larvae of *D. grandis* (Eaton) were covered with a growth of *Thiothrix* organisms, filamentous sulfide-oxidizing bacteria (Fig. 1). The bacteria seemed to have no lasting deleterious effect on the mayflies, as the latter changed into normal adults in late summer. Attempts to isolate this bacterium by methods previously used to isolate *Thiothrix* spp. from other locations (5) were unsuccessful.

Thin sections of the *Thiothrix* sp. provided evidence that some of its trichomes could be hosts to at least three morphologically distinct types of bacteria. These bacteria multiplied within the *Thiothrix* sp. while destroying the cytoplasm of the host. Attempts to obtain these parasites in pure cultures were unsuccessful, and they were designated simply as morphotypes 1 to 3. Some of their distinguishing features are shown in Table 1 and in Fig. 2 and 3.

The parasites appeared to have gram-negative cell wall structure, and they could be found in any cell along the length of the trichome, from a basal cell attached to the insect (Fig. 2A) to the terminal cell at the open end of the sheath (Fig. 2C). Bacteria of morphotypes 1 and 2 were capable of penetrating the septa between the cells in the *Thiothrix* trichomes (Fig. 2B and C and 3B), demonstrating that they were in the cytoplasm rather than in the periplasm. It is unclear whether morphotype 3 cells are periplasmic or

* Corresponding author.

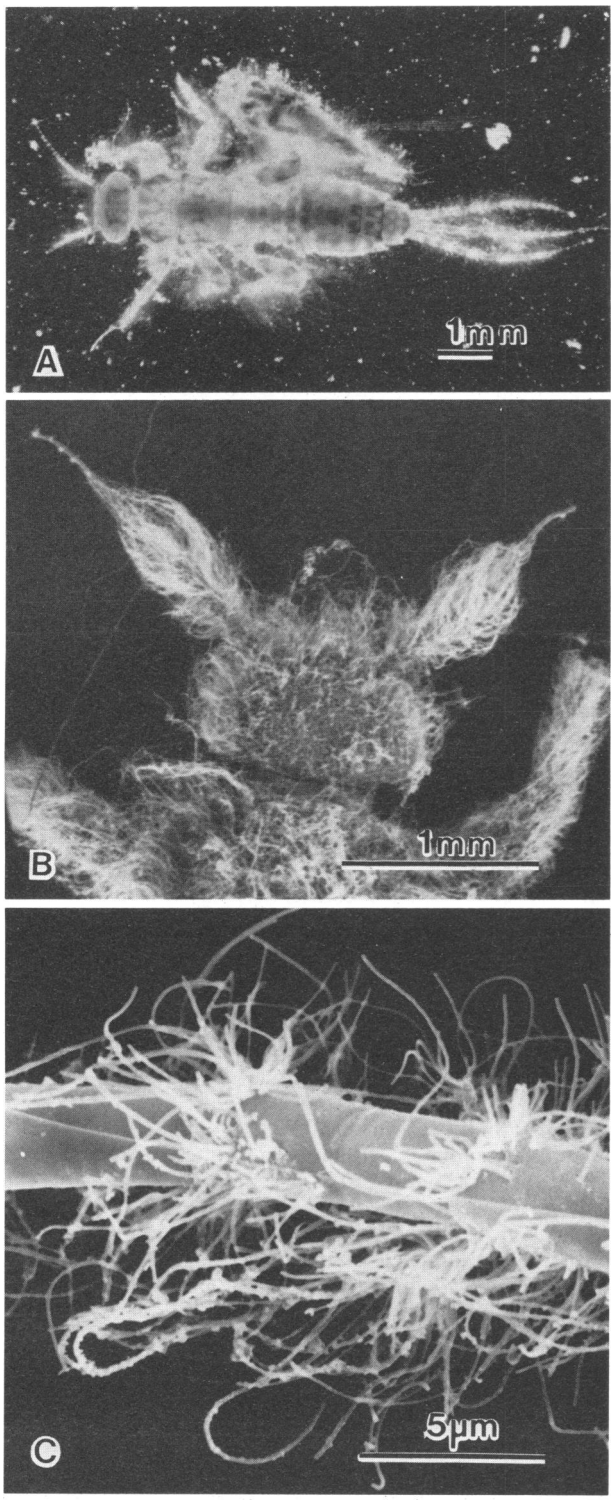

FIG. 1. Larvae of the mayfly *D. grandis* (Eaton) with attached *Thiothrix* trichomes. (A) Light micrograph of whole larva. (B) Scanning electron micrograph of anterior portion of larva. (C) Scanning electron micrograph of an anterior portion of the antenna.

cytoplasmic, although in Fig. 3E it appears that they probably reside in the periplasm.

DISCUSSION

Previously, there have been two genera of bacteria described as being able to penetrate a procaryotic host cell and obtain sustenance from it. *Bdellovibrio* organisms invade a wide range of host bacteria, but reproduce and remain within the periplasm of the host (1). Guerrero et al. (4) described the bacterium "*Daptobacter*" as being able to enter and reproduce in the cytoplasm of several genera of the family *Chromatiaceae* in which they utilize the cytoplasmic contents and leave only the cell wall of the host behind. "*Vampirococcus*" organisms (4) attack several *Chromatium* spp., but instead of entering the host cell, they attach to the outer surface and degrade the host cytoplasm from that location.

The three new organisms described in this paper apparently have the ability to penetrate the host cell wall, although no direct microscopic observation of this has been seen. At least two of these bacteria (morphotypes 1 and 2) also penetrate the cytoplasmic membrane and reproduce within the cytoplasm of the host. It is uncertain whether the third organism (morphotype 3) enters the cytoplasm or remains in the periplasmic space.

The mechanism(s) by which these parasitic bacteria enter and leave their host is unknown, but their presence within the cells of the host indicates that there must be some mechanism by which the parasites can penetrate both the sheath and the cell wall of the *Thiothrix* sp. for entrance. The act of penetrating the host does not cause lysis or other visible damage. The cytoplasmic parasites were able to penetrate the septa between the *Thiothrix* cells and thus spread among cells in the trichome.

There are at least two possible mechanisms by which the parasites could leave the host cell. Morphotypes 1 and 2 are able to penetrate the septa between cells in a trichome, and eventually the cytoplasmic contents as well as the septa are destroyed. This ability to destroy the septa may indicate that the parasites are able to pass through the end of the cell at the open end of a trichome and then leave through the unblocked open end of the sheath. While Fig. 3D is open to many interpretations, it may indicate that morphotype 3 cells are capable of producing a hole in the cell wall and sheath of the host from the inside, thus providing a means of escape.

The discovery of what appear to be three distinct bacteria that are internal parasites of *Thiothrix* organisms may indicate that internally parasitic bacteria are more prevalent than was previously thought. From the evolutionary standpoint it is of interest that all three of the bacteria ("*Daptobacter*" and morphotypes 1 and 2) known to reproduce in the cytoplasm of the host are parasites of bacteria that oxidize sulfide. Moreover, a criticism of the evolutionary theory of the origin of mitochondria and other organelles in eucaryotes by bacterial symbiosis has been the absence of phagocytosis, pinocytosis, or any other mechanism by which a procaryote can enter the cytoplasm of another procaryote (4). The ability of *Bdellovibrio* and our morphotype 3 organisms to enter the periplasm demonstrates the ability of bacteria to penetrate other bacteria, and the ability of "*Daptobacter*" and our morphotype 1 and 2 organisms to grow in the cytoplasm clearly shows that bacteria have the ability to penetrate and grow within other bacteria.

TABLE 1. Comparison of bacteria that are parasitic on sulfide-oxidizing bacteria

Organism	Source	Morphology	Size (μm)	Cell ends	Gram reaction[a]	Host	Location
"Daptobacter"[b]	Fresh water	Straight rod	0.5 by 1.5	Rounded	−	*Chromatiaceae*	Cytoplasm
Morphotype							
1	Sulfur spring	Straight rod	0.4 by 1.7–3.2	Rounded	−	*Thiothrix* sp.	Cytoplasm
2	Sulfur spring	Straight rod	0.4–0.5 by 1.0–4.5	Blunt	−	*Thiothrix* sp.	Cytoplasm
3	Sulfur spring	Straight rod	0.5 by 1.2[c]	Rounded	−	*Thiothrix* sp.	Periplasm

[a] Gram reaction is determined from cell wall morphology viewed by electron microscopy.
[b] Data from Guerrero et al. (4).
[c] Only a single longitudinal section has been found and measured.

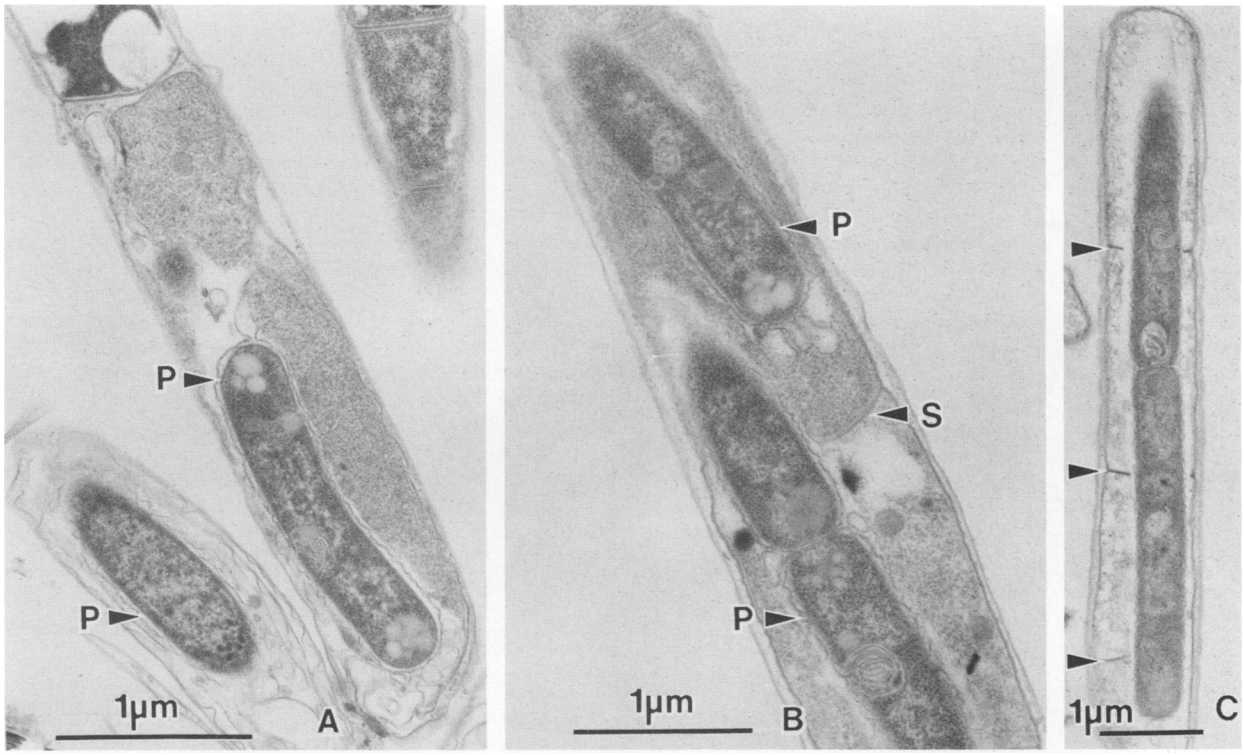

FIG. 2. Parasites of morphotype 1 within cells of a *Thiothrix* sp. (A) Single cells of the parasite (P). Both infected cells are basal cells of the trichomes and are attached to the surface of the larva. (B) A single cell and a dividing cell of the parasite. Note that the lower parasite is penetrating the septum (S) between two cells of the host. (C) Two cells of the parasite which have penetrated three septa (arrows) near the end of the host trichome. Note the absence of cytoplasm in the host.

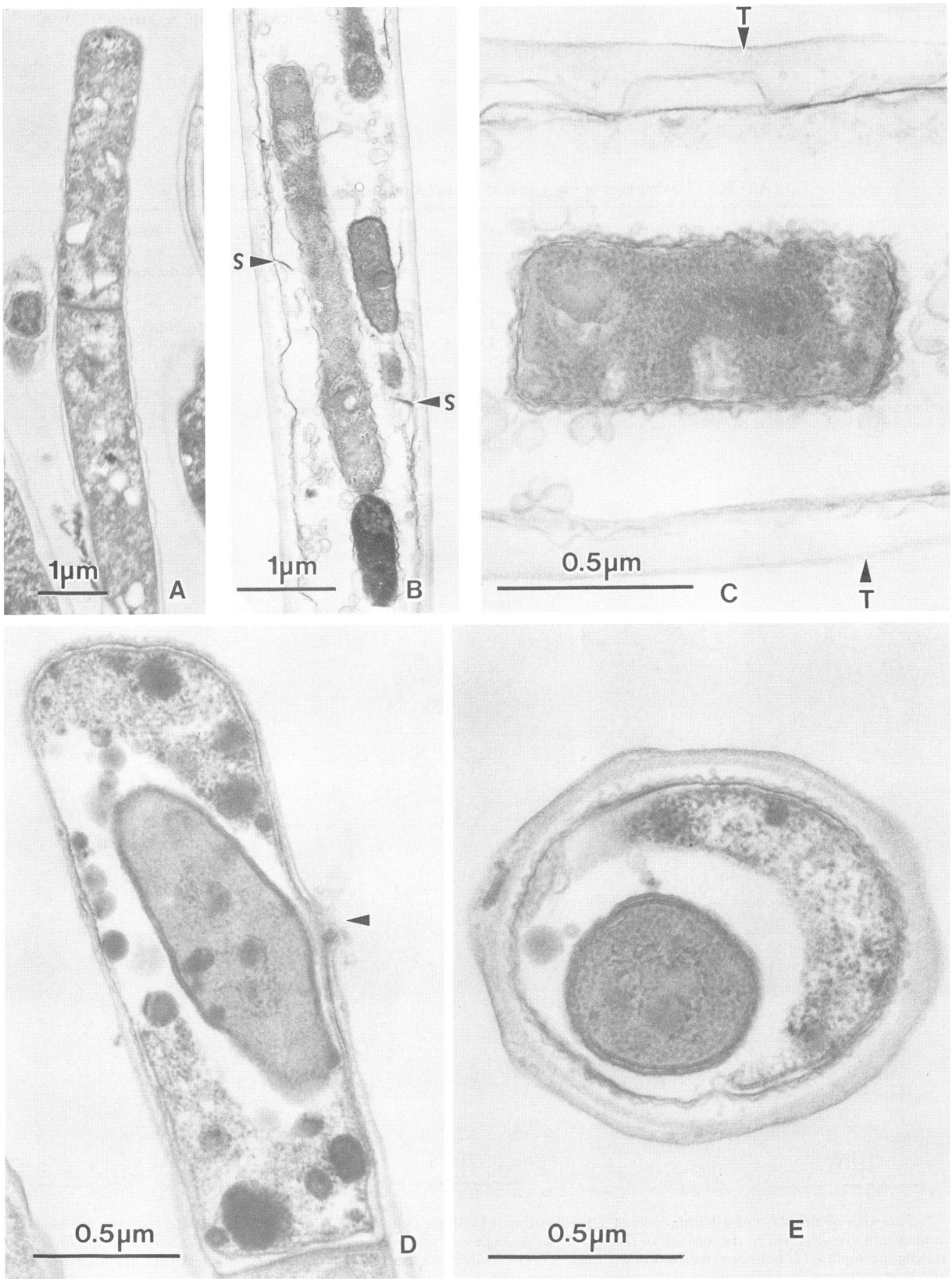

FIG. 3. Uninfected *Thiothrix* trichome and parasites of morphotypes 2 and 3. (A) Normal, uninfected trichome. (B) Parasites of morphotype 2. Note the remnants of destroyed septa (S) and the lack of cytoplasm in the host. The ends of this parasite are blunt. (C) Single cell of morphotype 2. Only the cell wall and sheath of the host *Thiothrix* sp. (T) remains. (D) Parasite of morphotype 3 within the terminal cell of a *Thiothrix* trichome. The arrow indicates a place where a weakness in the cell wall may have been produced and through which the parasite may emerge. (E) Transverse section of a parasite of morphotype 3. It appears that the cytoplasm has been pushed aside and that the parasite is in the periplasmic space.

ACKNOWLEDGMENTS

We thank Roy Bronander of Yavapai College for supplying laboratory space, R. Nelson for help with two micrographs, and the Louisiana State University College of Basic Sciences Electron Microscope Facility.

LITERATURE CITED

1. **Burnham, J. C., and S. F. Conti.** 1984. Genus *Bdellovibrio*, p. 118–124. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
2. **Burnham, J. C., and S. F. Conti.** 1984. Genus *Vampirovibrio*, p. 124. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
3. **Casida, L. E.** 1982. *Ensifer adhaerens* gen. nov., sp. nov.: a bacterial predator of bacteria in soil. Int. J. Syst. Bacteriol. **32**:339–345.
4. **Guerrero, R., C. Pedros-Alio, I. Esteve, J. Mas, D. Chase, and L. Margulis.** 1986. Predatory procaryotes: predation and primary consumption evolved in bacteria. Proc. Natl. Acad. Sci. USA **83**:2138–2142.
5. **Larkin, J. M.** 1980. Isolation of *Thiothrix* in pure culture and observation of a filamentous epiphyte on *Thiothrix*. Curr. Microbiol. **4**:155–158.
6. **Makkar, N. S., and L. E. Casida.** 1987. *Cupriavidus necator* gen. nov.: a nonobligate bacterial predator of bacteria in soil. Int. J. Syst. Bacteriol. **37**:323–326.
7. **McCurdy, H. D.** 1989. Order *Myxococcales*, p. 2139–2144. *In* J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 3. The Williams & Wilkins Co., Baltimore.
8. **Quinn, G. R., and V. D. B. Skerman.** 1980. *Herpetosiphon*—nature's scavenger. Curr. Microbiol. **4**:57–62.