APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1987, p. 1560–1570
0099-2240/87/071560-11$02.00/0

Vol. 53, No. 7

Ultrastructure of *Thiothrix* spp. and "Type 021N" Bacteria

TERRY M. WILLIAMS,[1] RICHARD F. UNZ,[1]* AND J. THOMAS DOMAN[2]

Laboratory of Environmental Microbiology, Department of Civil Engineering,[1] and Department of Molecular and Cell Biology,[2] The Pennsylvania State University, University Park, Pennsylvania 16802

Received 16 January 1987/Accepted 8 April 1987

The ultrastructural features of two groups of filamentous sulfur bacteria, *Thiothrix* spp. and an unnamed organism designated "type 021N," were examined by transmission electron microscopy. Negative staining of whole cells and filaments with uranyl acetate revealed the presence of tufts of fimbriae located at the ends of individual gonidia of *Thiothrix* sp. strain A1 and "type 021N" strain N7. Holdfast material present at the center of mature rosettes was observed in thin sections stained with ruthenium red. A clearly defined sheath enveloped the trichomes of two of three *Thiothrix* strains but was absent from "type 021N" filaments. The outer cell wall appeared more complex in "type 021N" strains than in *Thiothrix* isolates. Bulbs or clusters of irregularly shaped cells, often present in filaments of "type 021N" bacteria, appeared to result from crosswalls which formed at angles oblique to the filament axis. The multicellular nature of these sulfur bacteria was apparent in that only the cytoplasmic membrane and peptidoglycan layer of the cell wall were involved in the septation process. Sulfur inclusions which developed in the presence of sodium thiosulfate were enclosed by a single-layered envelope and located within invaginations of the cytoplasmic membrane.

Thiothrix spp. and a filamentous bacterium designated "type 021N" by Eikelboom (11) are colorless, sulfur-oxidizing bacteria which may form rosettes and gonidia and deposit sulfur when grown in the presence of sulfide or thiosulfate (16, 31). *Thiothrix* spp. inhabit sulfide-containing natural waters (17), and both *Thiothrix* and "type 021N" bacteria may develop in aerated wastewaters to the extent that the biological solids exhibit poor settling characteristics (activated sludge bulking [11, 19, 30]).

Recent studies have revealed important differences between "type 021N" strains and *Thiothrix* spp. in nutrition, physiology, and cultural morphology (30, 31; T. M. Williams, Ph.D. thesis, The Pennsylvania State University, University Park, Pa., 1985). Trichomes of "type 021N" bacteria display several unusual morphological features, including clusters of swollen cells (bulbs) (31), which appear similar to those described for *Leucothrix mucor* (26). Sheath formation, a characteristic of the genus *Thiothrix*, has been observed in the majority of strains isolated to date (16, 31). However, two types of filamentous bacteria, both believed to be *Thiothrix* spp., have been reported to exist in activated sludge: one is ensheathed, and another is of smaller diameter and lacks a sheath (12, 13).

Axenic cultures of *Thiothrix* and "type 021N" bacteria have only recently become available (15, 31); hence, little information is available about the ultrastructure of these filamentous sulfur bacteria. In this paper we describe the fine structure of representative strains of *Thiothrix* and "type 021N" bacteria isolated from activated sludge and comment on the relevant structure-function relationships.

MATERIALS AND METHODS

Bacteria and culture medium. *Thiothrix* and "type 021N" strains were isolated from activated sludge and have been described previously (31). *Thiothrix* sp. strain TH1 and "type 021N" strains N2 and N7 were made available by M. Richard, Colorado State University, Fort Collins. Stock cultures were maintained in liquid LTH mineral salts medium (31), which contained sodium lactate (1.0 g/liter), sodium thiosulfate (0.5 g/liter), and HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (0.01 M), final pH 7.5.

Electron microscopy. Ultrastructural studies were performed on washed cells from 2- to 3-day-old shake cultures developed on LTH medium at 25°C. Whole cells were negatively stained with 0.5% uranyl acetate for 30 s on Formvar-carbon-coated 400-mesh copper grids. The ruthenium red-glutaraldehyde-osmium tetroxide fixation procedure used for thin sectioning has been described elsewhere (31). Briefly, fixed specimens were dehydrated through a graded series of ethanol and embedded in Spurr epoxy resin. Thin sections were collected on 300-mesh uncoated copper grids and stained with uranyl acetate and lead citrate. Grids were examined with a Philips EM-300 transmission electron microscope at 60 kV.

RESULTS

General morphological characteristics. The overall appearance and characteristics of the *Thiothrix* and "type 021N" trichomes are illustrated in Fig. 1. Individual cells within filaments of both *Thiothrix* and "type 021N" bacteria were separated by readily visible crosswalls and contained compact clusters of densely stained material, presumably ribosomes. Three morphologically distinct types of intracellular inclusions were recognized in thin sections: sulfur, poly-β-hydroxybutyrate (PHB), and polyphosphate (Fig. 1; also see Fig. 4 and 6).

Trichomes of *Thiothrix* sp. strains A1 (Fig. 1a and b) and Q (Fic. 1c) were composed of rod-shaped cells. Sheath material was evident in sections of *Thiothrix* sp. strain Q, but was never observed in *Thiothrix* sp. strain A1.

Individual cells within filaments of "type 021N" strain N7 appeared cylindrical to barrel-shaped (Fig. 1d and e), whereas portions of "type 021N" strain N2 trichomes (Fig. 1f) more closely resembled the rod-shaped cells of *Thiothrix* spp. "Type 021N" bacteria did not form sheaths.

Incompletely formed septa were visible in actively dividing cells along the length of *Thiothrix* and "type 021N"

* Corresponding author.

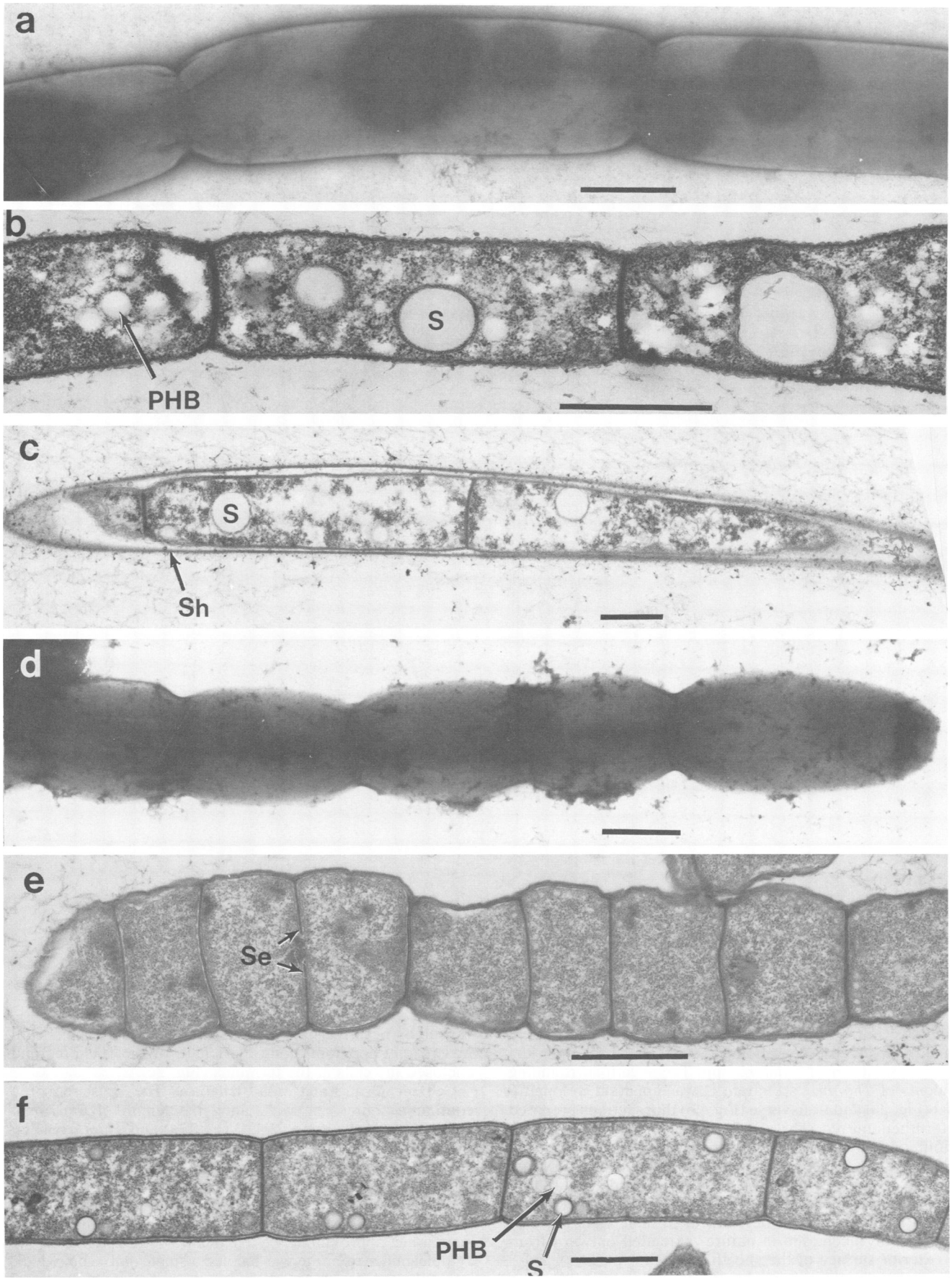

FIG. 1. General ultrastructural characteristics of trichomes of *Thiothrix* and "type 021N" bacteria. *Thiothrix* sp. strains A1 (a, b) and Q (c); "type 021N" strains N7 (d, e) and N2 (f). Abbreviations: Sh, sheath; Se, septum; S, sulfur inclusion. Bars, 1.0 μm.

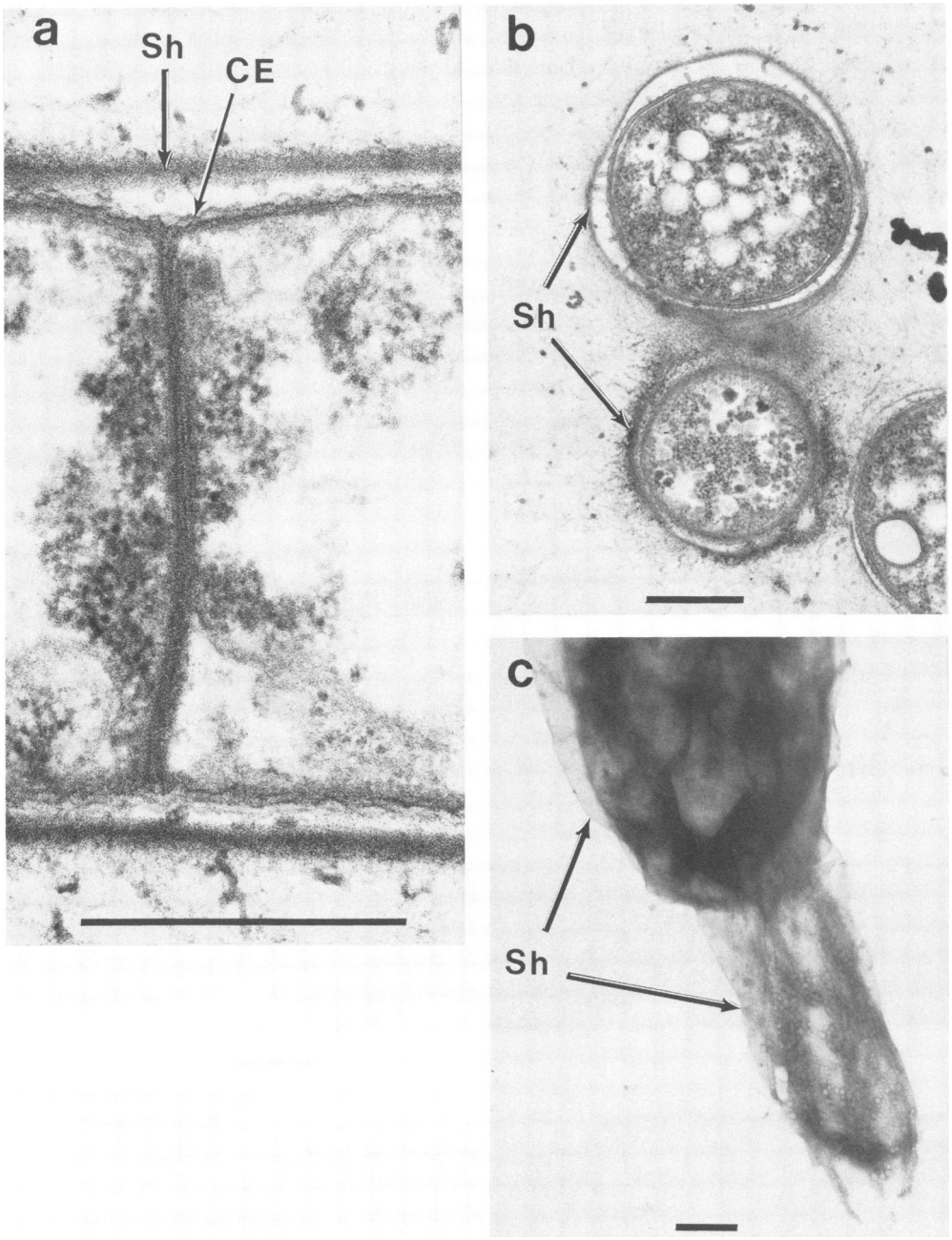

FIG. 2. Sheath characteristics in *Thiothrix* sp. strains Q (a, b) and TH1 (c). Abbreviations: Sh, sheath; CE, cell envelope. Bars, 0.5 μm.

trichomes (Fig. 1e). Flagella were not detected in negatively stained whole-cell preparations.

Sheath structure of *Thiothrix* spp. The sheath surrounding filaments of *Thiothrix* sp. strain Q was apparent in longitudinal (Fig. 2a) and transverse (Fig. 2b) thin sections prepared with ruthenium red. The relative proximity of the sheath to the outer cell wall varied, and in many cases a large space (77 to 135 nm) was observed. The sheath of *Thiothrix* sp. strain Q was approximately 15 to 60 nm in width and appeared, in certain regions, to consist of several separate layers (Fig. 3e). An additional dense network of fibrillar material, presumably polysaccharide in nature, extended outward from the exterior surface of the sheath (Fig. 2b).

Sheath material produced by *Thiothrix* sp. strain TH1 was viewed by negative staining with uranyl acetate. The sheath was readily apparent along the length of the trichome and enclosed the cells at the terminus (Fig. 2c).

Cell membrane and wall structure. The presence of a continuous outer cell wall along the length of individual filaments of both "type 021N" (Fig. 3a) and *Thiothrix* (Fig. 2a) strains illustrates the multicellular nature of these filamentous organisms. A comparison of the fine structure of the cell envelope components in *Thiothrix* and "type 021N" strains is presented in Table 1. The cytoplasm in both kinds of sulfur bacteria was enclosed within a trilaminar unit membrane, approximately 5 to 8 nm in width. Exterior to the cytoplasmic membrane was located a single (4 to 6 nm wide), intensely staining layer (Fig. 3c, d, and e), which corre-

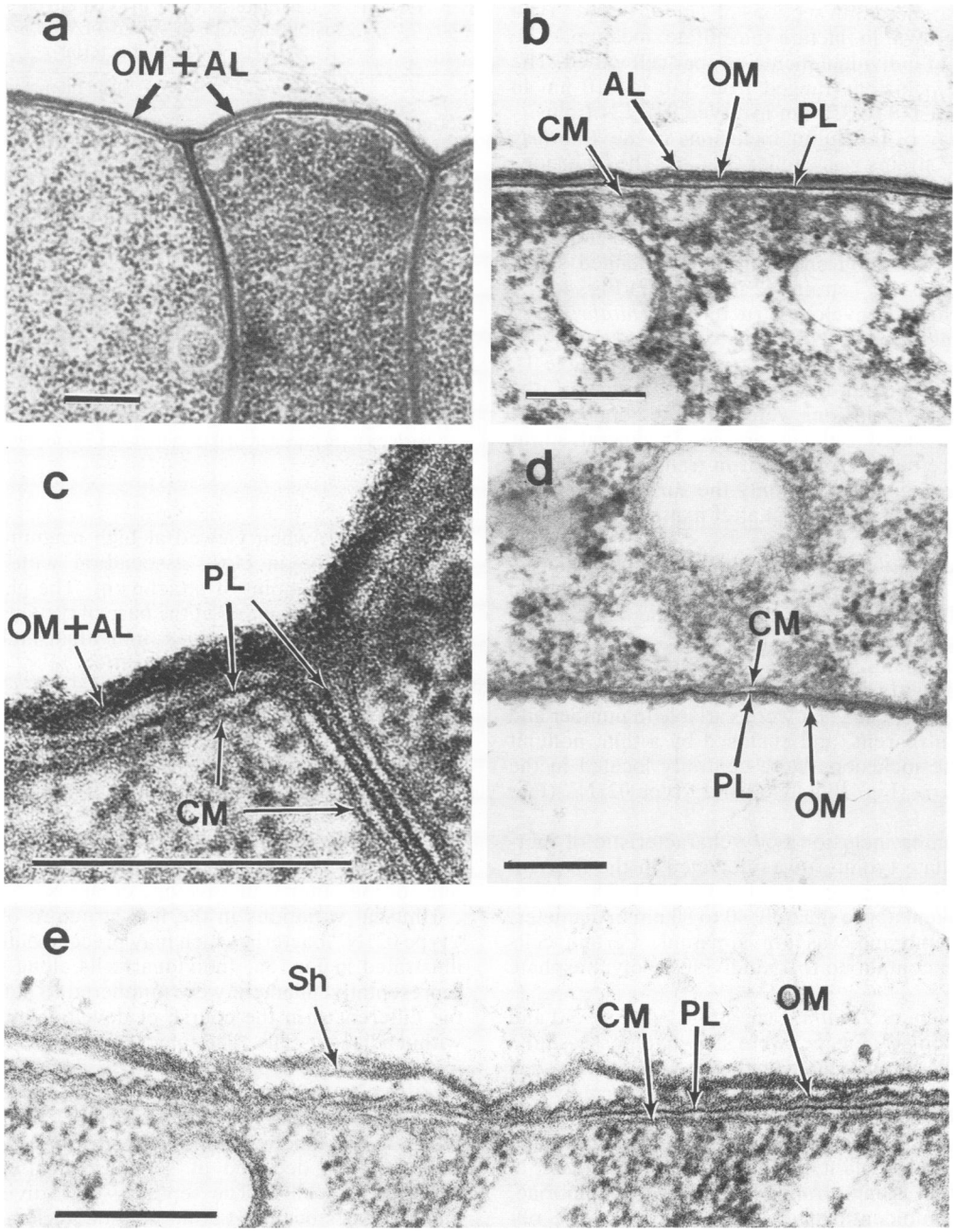

FIG. 3. Cell membrane and envelope structure in strains of *Thiothrix* and "type 021N" bacteria. "Type 021N" strains N7 (a, b) and N2 (c); *Thiothrix* strains A1 (d) and Q (e). Abbreviations: CM, cytoplasmic membrane; PL, peptidoglycan layer of cell wall; OM, outer membrane; AL, additional outer layers; Sh, sheath. Bars, 250 nm.

sponds to the peptidoglycan layer of the gram-negative cell wall. The next outer layer of the cell envelope consisted of a double-track membrane which was highly convoluted in appearance and similar in width to the cytoplasmic membrane of the bacteria (Table 1).

The outer cell wall structure of "type 021N" strains N7 (Fig. 3b), N2 (Fig. 3c), and A (see Fig. 8a) differed from that observed in the *Thiothrix* isolates (Fig. 3d and e) by the presence of one or more thin, dense layers located external to the outer wall membrane. The total thickness of the entire cell wall complex in "type 021N" strains (29 to 43 nm) was considerably greater than in the *Thiothrix* isolates (18 to 27 nm). Cell division in the filamentous sulfur bacteria was apparently initiated via transverse septum formation, which occurred perpendicular to the longitudinal axis of the filaments. Enlargement of a typical section through the septal region of adjacent cells of "type 021N" strain N2 (Fig. 3c) revealed that crosswall formation resulted from ingrowth of the cytoplasmic membrane and peptidoglycan layer only; the outer layers of the cell envelope were not involved in this process.

Sulfur inclusion envelope morphology. Sulfur inclusions

were readily apparent in thin sections of *Thiothrix* and "type 021N" strains grown in lactate-thiosulfate medium. The number and size of individual inclusions per cell varied. The diameter of the inclusions ranged from 110 to 810 nm in *Thiothrix* spp. and 148 to 350 nm in "type 021N" strains.

The morphology of the sulfur inclusions in the *Thiothrix* and "type 021N" strains was similar (Fig. 4). The inclusions were located either adjacent to the cell septa or along the periphery of the wall. Invaginated pockets of the cytoplasmic membrane were apparent (Fig. 4b, arrow). An elongated and extensive invagination enclosing a well-defined sulfur inclusion in "type 021N" strain N2 is shown in Fig. 4d.

The sulfur inclusion envelope structure in *Thiothrix* spp. (Fig. 4a and e) and "type 021N" strain N2 (Fig. 4d and f) was observed as a single, thin, densely stained layer (Table 1), separate from but bound by the unit membrane of the cytoplasm; thus, the inclusions were within the boundary of the cell wall but external to the cytoplasm. In general, sulfur inclusions appeared as vacant, electron-transparent spaces, and in certain sections (Fig. 4c) only the surrounding cytoplasmic membrane remained intact after fixation and ethanol dehydration.

Examination of whole cells by scanning electron microscopy and X-ray energy-dispersive microanalysis confirmed that sulfur was the principal component of the sulfur inclusions (unpublished data).

Other inclusion bodies. The most frequently encountered inclusions present within the cytoplasm of the sulfur bacteria resembled PHB granules. They were variable in number and size, electron transparent, and enclosed by a thin, nonunit membrane. These inclusions were centrally located in the cells of all *Thiothrix* (Fig. 1b and 4a) and "type 021N" (Fig. 4b and f) strains.

A densely staining inclusion body characteristic of inorganic polyphosphate (volutin) was detected in the filamentous sulfur bacteria (Fig. 4c; see also Fig. 6b). They were centrally located and approximately 40 to 80 nm in diameter. An enclosing membrane was not apparent. Certain cells were observed to contain up to 15 individual polyphosphate inclusions.

Fimbriae. Gonidia of *Thiothrix* sp. strain A1 (Fig. 5a) and "type 021N" strain N7 (Fig. 5c) were shown to possess tufts of polar to subpolar fimbriae in negatively stained whole-cell preparations. Individual fimbriae were approximately the same diameter in *Thiothrix* sp. strain A1 (6 to 9 nm) and "type 021N" strain N7 (7 to 9 nm). The length of the appendages was not constant, and strands 3 μm or greater in length were noted. Long strands of intertwined fimbriae, together with an unidentified globular material, were observed occasionally at the base of single filaments (Fig. 5b).

Rosette structure. The ultrastructural features of rosettes formed by *Thiothrix* and "type 021N" strains were similar (Fig. 6). Thin sections through the center of mature rosettes revealed an extensive network of holdfast material surrounding the basal cells of the filaments (Fig. 6a and b). The holdfasts were intensely stained in the presence of ruthenium red.

Certain cells composing the basal portions of *Thiothrix* and "type 021N" rosettes (Fig. 6a and b, respectively) were noticeably greater in overall length and width than other cells along the interior of the filaments. Certain of these enlarged basal cells were observed to be actively dividing.

Negatively stained whole cells of *Thiothrix* sp. strain A1 revealed an amorphous material at the hub of the rosette. This material, when viewed at high magnification (Fig. 7), appeared to be in close association with the individual filaments at the point of attachment. The globular material resembled that observed at the base of the single, unattached filament in Fig. 5b; however, individual fimbriae were not apparent at the center of the rosette.

TABLE 1. Characteristics of the cell envelope and sulfur inclusion envelope in strains of *Thiothrix* and "type 021N" bacteria[a]

Ultrastructural feature	Diam (nm) of component			
	Thiothrix sp.		"Type 021N"	
	A1	Q	N2	N7
Cytoplasmic membrane (double layer)	4–5	6–8	7–8	6–8
Inner wall layer (peptidoglycan)	4–5	4–5	4–5	5–6
Outer wall membrane (double layer)	5–7	6–9	7–8	6–8
Cell wall complex (all layers)	20–24	18–27	30–43	29–36
Sulfur inclusion envelope (single)	3–5	4–5	3–4	5–8

[a] Bacteria were grown in shaken culture in LTH medium, 25°C, 2 to 3 days of incubation.

Unusual features of "type 021N" bacteria. Clusters of large or swollen cells (bulbs) frequently observed in cultures of "type 021N" bacteria were apparent in thin sections. Enlargement of a section obtained through the center of a bulb in "type 021N" strain A (Fig. 8b) revealed a nonlinear arrangement of cells, with crosswalls formed at odd angles to the longitudinal axis of the filament. The individual cells constituting the bulbs were bound entirely within the confines of the outer wall envelope complex.

Unusual variations in the fine structure of crosswalls of "type 021N" bacteria which may precede bulb formation are illustrated in Fig. 8a. Individual cells along the length of a representative filament were numbered to aid in recognizing the differences in the course of development of crosswalls within adjacent cells. The typical process of division involving ingrowth of septa is evident in cell 1. Cell division at locations 2 and 3 was apparently initiated by a single septum from only one part of the cell wall. Cells 4 and 7 were nondividing. Three incompletely formed septa (Fig. 8a, arrows) were detected at separate locations around the periphery of cell 5. The septum which divided cell 6 into unequal parts occurred at an oblique angle and was closely associated with the midportion of the crosswall between cells 6 and 7. Given the above, bulb formation of the type observed in Fig. 8b would most likely arise in the vicinity of cell 5 or 6.

Another unusual ultrastructural feature encountered occasionally in "type 021N" cells was the presence of an additional double-layered membrane located either centrally within the cytoplasm (Fig. 8c, arrows) or along the periphery of the cell boundary (Fig. 8d, arrows). In cross section, these structures were typically visible only on one side of the cell. When present, this unidentified membrane complex occurred internal to the cytoplasmic membrane. Its significance is not known.

DISCUSSION

In previous studies with filamentous sulfur bacteria, including *Thiothrix* spp. (1, 16), *Thioploca* sp. (18), and *Beg-*

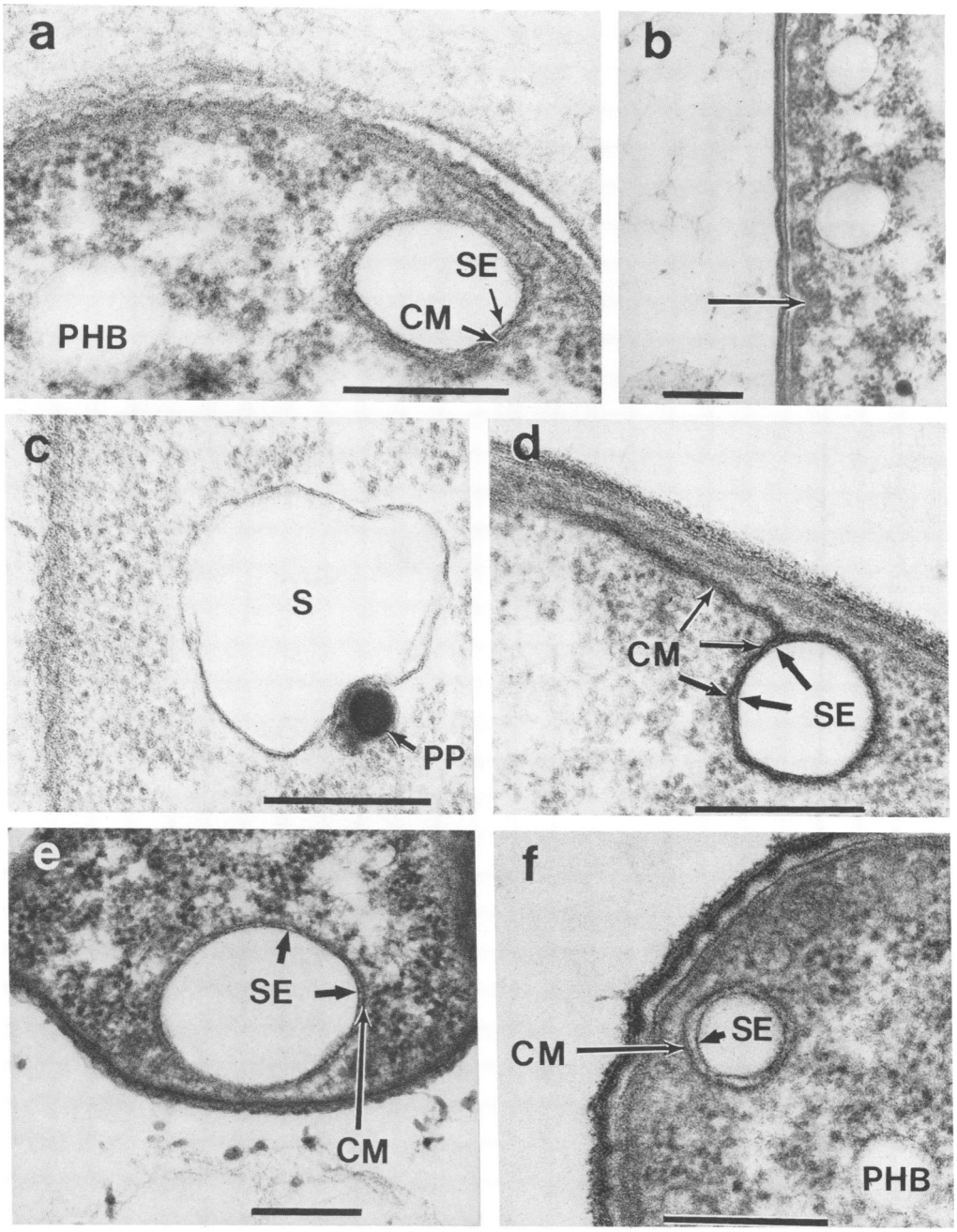

FIG. 4. Sulfur inclusions in "type 021N" bacteria and *Thiothrix* spp. *Thiothrix* sp. strains Q (a, c) and A1 (e); "type 021N" strains N7 (b) and N2 (d, f). Abbreviations: S, sulfur inclusions; SE, sulfur inclusion envelope; CM, cytoplasmic membrane; PP, polyphosphate. Arrow in panel b indicates invagination of the CM. Bars, 250 nm.

giatoa spp. (17, 18, 20), sulfur inclusions were located within invaginated pockets of the cell membrane; however, well-defined inclusion envelopes were not clearly visible, possibly due to different fixation procedures. Conversely, sulfur inclusions present in unicellular sulfur bacteria, i.e., *Thiovulum* (33) and *Chromatium* (21), were enclosed by a single layer only and were not external to the cytoplasm.

The sulfur inclusion envelope-cytoplasmic membrane complex observed in our strains of *Thiothrix* and "type 021N" bacteria was most similar to that reported and described for *Beggiatoa alba* strains B18LD, B25RD, and L1401-13 by Strohl et al. (27). The inclusion envelope was a single layer (4 to 5 nm in width) in these *Beggiatoa* isolates; however, a pentalaminar structure (12 to 14 nm in width) was reported for *B. alba* B15LD (28).

The specialized arrangement of the sulfur inclusions present in the filamentous sulfur bacteria suggests an important structure and function relationship for the oxidation of sulfide and thiosulfate and sulfur inclusion formation. The location of the inclusions within the periplasmic space may be of consequence for the bacteria in several ways. Oxidation of sulfide at the outer surface of the plasma membrane, adjacent to the sulfur inclusion, may (i) establish a proton gradient necessary for ATP synthesis and (ii) reduce the

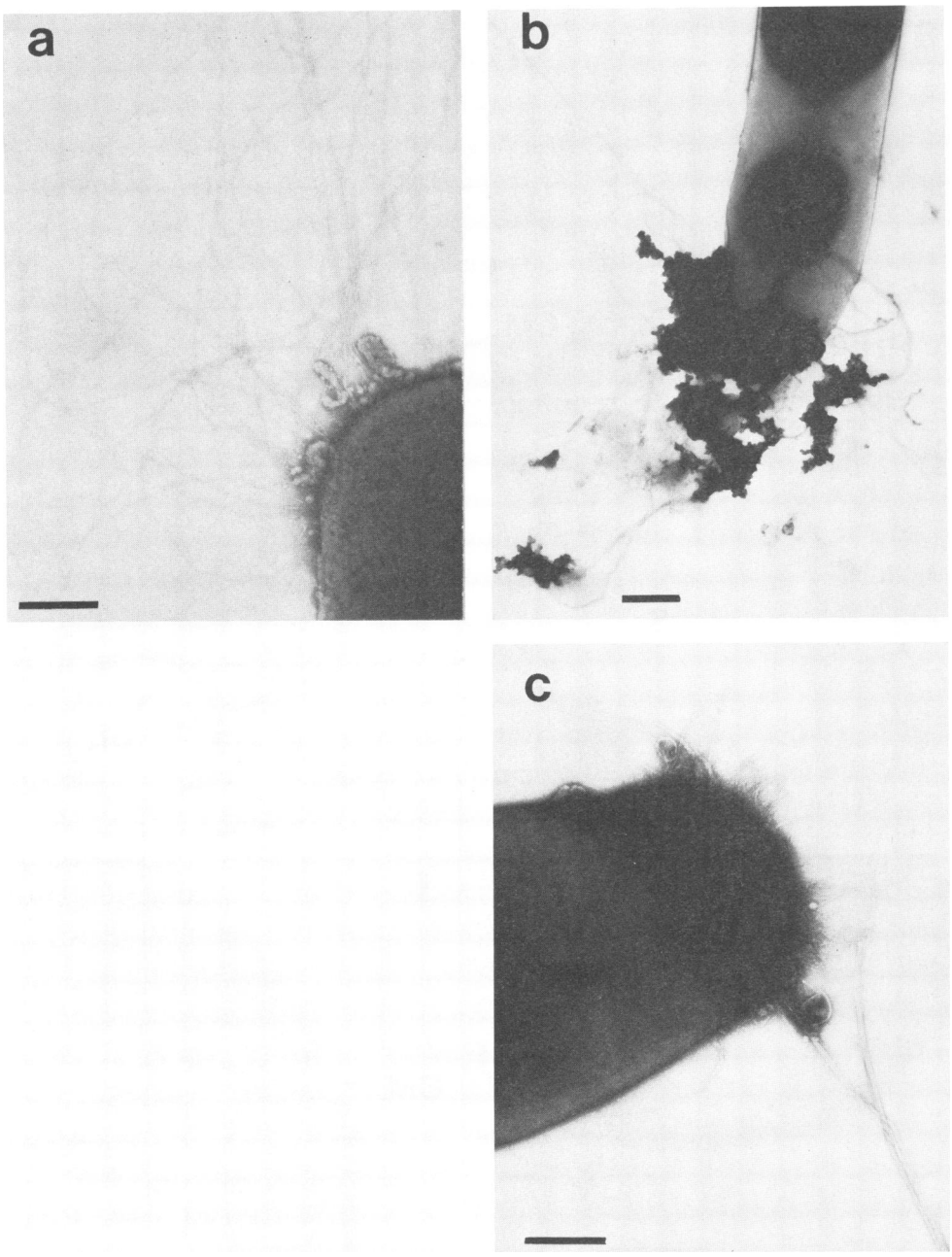

FIG. 5. Negatively stained preparations of fimbriae present in the filamentous sulfur bacteria. Gonidia of *Thiothrix* sp. strain A1 (a) and "type 021N" strain N7 (c); Globular material and entangled fimbriae at the terminus of a single filament of *Thiothrix* sp. strain A1 (b). Bars, 0.5 μm.

potential for sulfide toxicity within the cytoplasm (17). Williams and Unz (31) reported that growth of *Thiothrix* and "type 021N" bacteria in the presence of sulfide or thiosulfate resulted in sharp decreases in the pH of the culture medium. An additional benefit to the cell would be that acidic end products (H_2SO_4) from the oxidation of reduced sulfur compounds or intact sulfur inclusions would be generated and excreted outside the cytoplasm.

It has been suggested that the inclusion membrane enclosing the sulfur inclusions in *Chromatium* spp. may contain binding sites for enzymes responsible for the metabolism of sulfur stores (24). Degradation of intracellular lipid reserves in bacteria is known to involve enzymes associated with the membrane which encloses PHB granules (25). The enzymes responsible for oxidation of stored sulfur in the filamentous bacteria might similarly be associated with the sulfur inclusion membrane.

The cell wall structure of the present *Thiothrix* isolates was similar to that of other *Thiothrix* spp. (1, 16) and, in general, to *L. mucor* (2, 26). The overall appearance of the cell envelope of the *Thiothrix* spp. was typical of gram-negative bacteria (9).

By comparison, "type 021N" bacteria differed from the *Thiothrix* strains by the presence of one or more additional layers located exterior to the outer membrane. Other genera of filamentous sulfur bacteria possess a complex outer wall

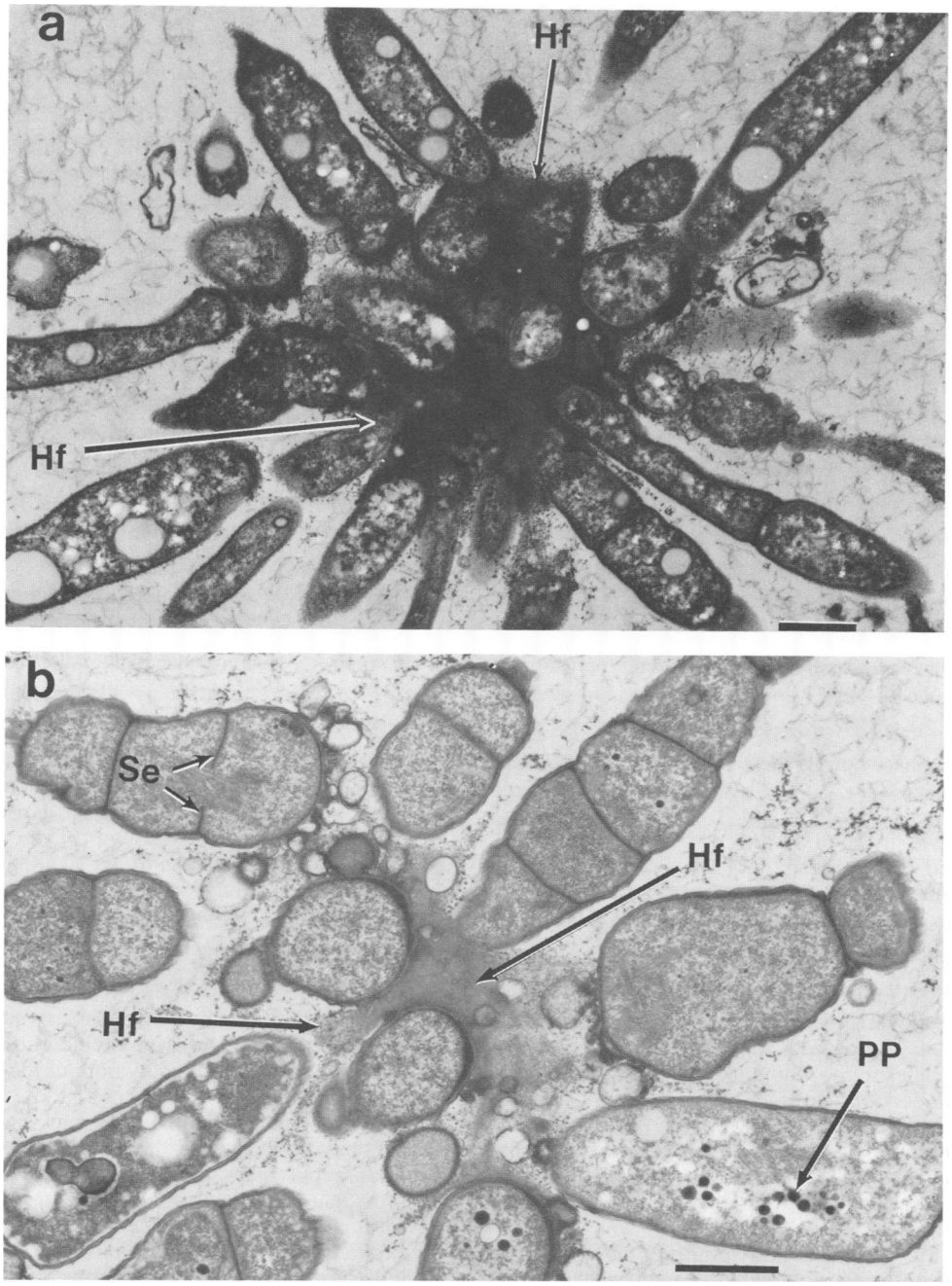

FIG. 6. Thin sections through rosettes in *Thiothrix* and "type 021N" bacteria. *Thiothrix* sp. strain A1 (a) and "type 021N" strain N7 (b). Abbreviations: Hf, holdfast material; PP, polyphosphate; Se, cell septum. Bars, 1.0 μm.

arrangement as well. The cell wall structure of *Thioploca ingrica* consists of a total of five separate layers external to the cytoplasmic membrane (18). *Beggiatoa* spp. may contain from three to five distinct cell wall layers, depending on the specific strain examined and the fixation procedure used (18, 28).

A variety of aquatic bacteria also possess additional outer cell wall layers (9, 23). Costerton et al. (9) suggested that these external layers may afford an advantage to the organisms in highly competitive environments by providing a selective barrier against hostile elements, e.g., antibiotics and exoenzymes. Interestingly, "type 021N" bacteria were shown to be less sensitive than *Thiothrix* strains to polymyxin B (31), an antibiotic active against gram-negative bacteria. The presence of protective layers in filamentous sulfur bacteria may contribute to resistance against disinfectants, e.g., chlorine, used to correct activated sludge bulking.

"Type 021N" bacteria frequently exhibit vacant regions or empty cuffs at the ends of the filaments when viewed by phase-contrast microscopy (11, 31). These bacteria did not possess ultrastructural features resembling a typical sheath, and the empty sleeves or cuffs may represent visible remnants of the thick, closely associated outer layers of the cell wall, which run continuously along the length of the

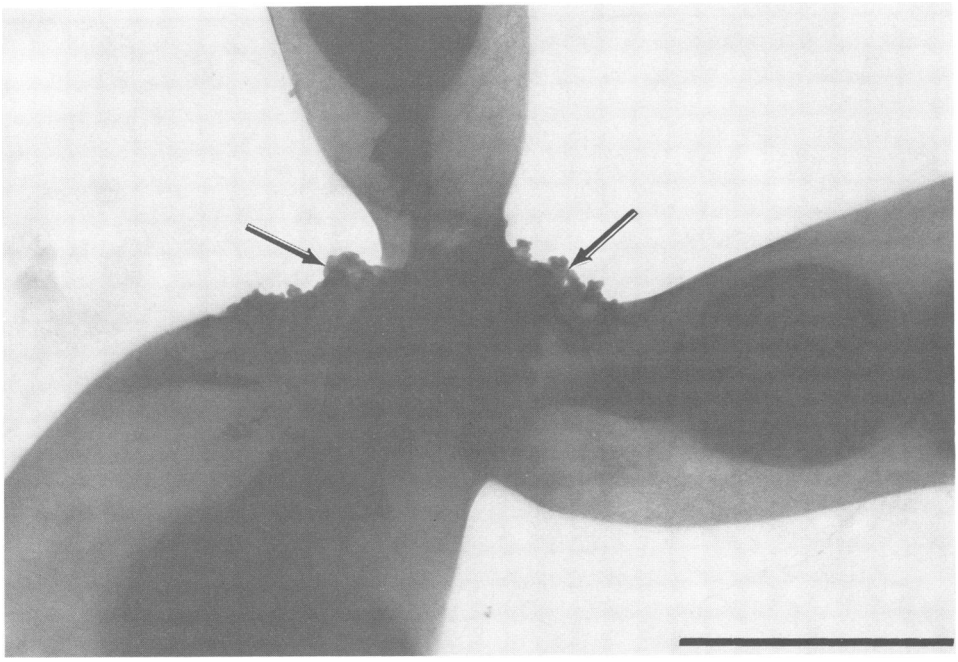

FIG. 7. Negatively stained preparation of a rosette formed by *Thiothrix* sp. strain A1. High magnification of globular material (arrows) associated with the rosette center. Bar, 1.0 μm.

trichomes. Similar observations have been reported in other filamentous organisms (23).

Cell division in both *Thiothrix* and "type 021N" bacteria occurred by transverse septation and was indistinguishable from that described for other genera of filamentous bacteria, including *Leucothrix* (2, 26), *Beggiatoa* (17, 18, 28), and *Thioploca* (18). In these multicellular organisms, only the inner peptidoglycan layer and not the outer layer(s) participated in septum formation.

Bulblike structures, observed frequently in wet-mount preparations of "type 021N" trichomes (31), were apparent in thin sections and appeared to result from crosswall formation at odd angles to the filament axis. Brock and Conti (2) failed to obtain sections through bulbs in *L. mucor*, but considered these structures to result from the fusion of cells, which occurred in knots along the filament. The mechanism of bulb formation in *L. mucor* was described by Snellen and Raj (26) and appeared similar to that observed in this study for the "type 021N" bacteria.

The presence of a sheath surrounding filaments of *Thiothrix* spp. was described originally by Winogradsky (32) and has been recognized repeatedly (1, 5, 15, 16, 31). Reichenbach (23), however, failed to acknowledge *Thiothrix* spp. as being among the ensheathed gliding bacteria. Sheath formation was observed in only two of the three *Thiothrix* strains examined in this paper. The sheath produced by these *Thiothrix* isolates appeared similar to that of *Thiothrix nivea* JP2 (16) and consisted of several discrete layers of variable width. Trichomes of *Thiothrix* spp. collected from a sulfur spring and studied by Bland and Staley (1) formed a sheath which was densely stained and appeared uniform (60 to 70 nm) in thickness. Environmental factors are known to influence sheath thickness. For example, the sheath produced by the filamentous bacterium *Sphaerotilus natans* may vary in diameter according to the nutrient content of the culture medium (10, 14).

The absence of a sheath in *Thiothrix* sp. strain A1 may be due to (i) limitations inherent in this strain for synthesis of this material, (ii) the requirement for specific culture conditions not provided herein, or (iii) loss of this capacity through mutation acquired during prolonged subculturing. It should be noted that the *Thiothrix* filaments present in the raw sample from which strain A1 was isolated were not ensheathed. Other investigators have reported a type of *Thiothrix* sp. in activated sludge which did not possess a sheath (12, 13).

All of the *Thiothrix* strains isolated and described by Williams and Unz (31), as well as several "type 021N" isolates, developed rosettes in axenic culture. Filaments at the center of rosettes formed by both of these sulfur bacteria were surrounded by an amorphous holdfast material which was densely stained by ruthenium red, suggesting the presence of acidic polysaccharides (8), although other types of polyanionic polymers are possible (3). Holdfast material similar to that described in this study has also been demonstrated in association with rosette formation by other types of bacteria (7), including *L. mucor* (2), *Hyphomicrobium* sp. (6), and *Rhizobium* sp. (29). Rosette formation in *Thiothrix* spp. may not be a stable characteristic. Larkin (personal communication) noted that repeated transfer of his axenic *Thiothrix* cultures resulted in the inability of certain strains to develop rosettes.

Gonidial cells of both *Thiothrix* and "type 021N" bacteria were shown to possess tufts of polar fimbriae. Fimbriae have also been reported in strains of *T. nivea* (16) and other nonsulfur gliding bacteria (4). Similar types of appendages have not been reported in *Leucothrix* (2, 4, 26) or *Beggiatoa* (4, 17, 18, 28) isolates. Bacterial fimbriae have been suggested to function in aspects of gliding motility, attachment to surfaces, and cell-to-cell adhesion (4, 7, 22). In *Thiothrix* spp., gonidia which aggregate to form rosettes have been shown to be oriented with the fimbriated ends towards the center of the rosette (17). This orientation suggests an important role for fimbriae in the development of colonial

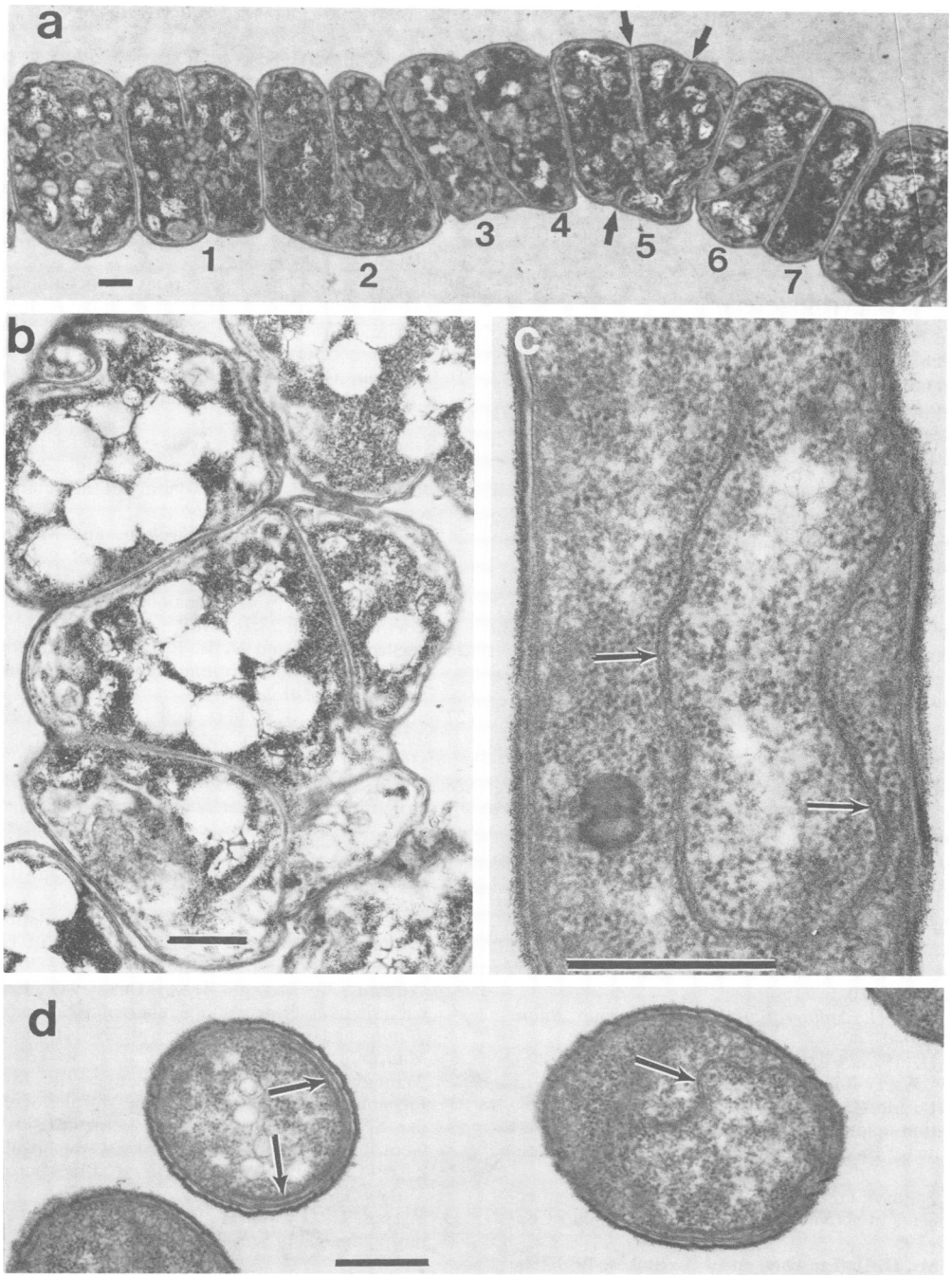

FIG. 8. Unusual morphological features observed in "type 021N" bacteria. Atypical septum formation (arrows) in strain A (a, b); strain N2 (c, d) with unidentified internal membrane structures (arrows). Bars, 0.5 μm.

growth forms by *Thiothrix* and perhaps "type 021N" bacteria.

Thiothrix spp. occur in a variety of natural waters, primarily in a sessile growth habit (5, 17, 32). The natural habitat of "type 021N" bacteria is not known, beyond its occurrence in waste treatment systems (11, 19, 30). The ability of *Thiothrix* and "type 021N" bacteria to attach to surfaces in nature is probably aided by the presence of specialized structures (fimbriae and extracellular fibrillar material). Merkel (19) provided evidence of a holdfast at the point of attachment of *Thiothrix* filaments to stalks of ciliated protozoa in activated sludge. Dondero (10) suggested that holdfasts may be produced by microorganisms after adhesion has been established by some other means. The initial step in attachment to solid surfaces and in the formation of rosettes by *Thiothrix* and "type 021N" bacteria may involve physical contact and intertwining of the fimbriae with the substratum or with other fimbriated cells. Once secured, secretion of the holdfast material by the cells would then provide the adhesion necessary to allow filaments to remain attached. The large, basal cells located at the hub of rosettes in *Thiothrix* and "type 021N" strains may provide additional structural support for these aggregates.

Determination of the composition of the cell envelope,

sheath, and holdfast material and the influence of pertinent environmental factors on their development is needed to better understand the ecological role of the filamentous sulfur bacteria.

ACKNOWLEDGMENT

This investigation was supported by the National Science Foundation, Division of Civil and Environmental Engineering, Environmental and Water Quality Engineering Program (grant CEE-7918919).

LITERATURE CITED

1. **Bland, J. A., and J. T. Staley.** 1978. Observations on the biology of *Thiothrix*. Arch. Microbiol. **117**:79–87.
2. **Brock, T. D., and S. F. Conti.** 1969. Electron microscopic studies on *Leucothrix mucor*. Arch. Mikrobiol. **66**:79–90.
3. **Brooker, B. E., and R. Fuller.** 1976. Demonstration of a carbohydrate layer involved in the attachment of lactobacilli to the chicken crop epithelium, p. 87–100. *In* R. Fuller and D. W. Lovelock (ed.), Microbial ultrastructure. Academic Press, Inc., New York.
4. **Burchard, R. P.** 1981. Gliding motility of prokaryotes: ultrastructure, physiology, and genetics. Annu. Rev. Microbiol. **35**:497–529.
5. **Caldwell, D. E., S. J. Caldwell, and J. M. Tiedje.** 1975. An ecological study of the sulfur-oxidizing bacteria from the littoral zone of a Michigan lake and a sulfur spring in Florida. Plant Soil **43**:101–114.
6. **Conti, S. F., and P. Hirsch.** 1965. Biology of budding bacteria. III. Fine structure of *Rhodomicrobium* and *Hyphomicrobium* spp. J. Bacteriol. **89**:503–512.
7. **Corpe, W. A.** 1980. Microbial surface components involved in adsorption of microorganisms onto surfaces, p. 105–144. *In* G. Bitton and K. C. Marshall (ed.), Adsorption of microorganisms to surfaces. John Wiley & Sons, Inc., New York.
8. **Costerton, J. W.** 1980. Some techniques involved in study of adsorption of microorganisms to surfaces, p. 403–423. *In* G. Bitton and K. C. Marshall (ed.), Adsorption of microorganisms to surfaces. John Wiley & Sons, Inc., New York.
9. **Costerton, J. W., J. M. Ingram, and K. J. Chen.** 1974. Structure and function of the cell envelope of gram-negative bacteria. Bacteriol. Rev. **38**:87–110.
10. **Dondero, N. C.** 1975. The *Sphaerotilus-Leptothrix* group. Annu. Rev. Microbiol. **29**:407–428.
11. **Eikelboom, D. H.** 1975. Filamentous organisms observed in activated sludge. Water Res. **9**:365–388.
12. **Eikelboom, D. H., and H. J. J. van Buijsen.** 1981. Microscopic sludge investigation manual. IMG-TNO report no. A94a. TNO Research Institute for Environmental Hygiene, Delft, The Netherlands.
13. **Farquhar, G. J., and M. C. Boyle.** 1971. Occurrence of filamentous microorganisms in activated sludge. J. Water Pollut. Control Fed. **43**:779–798.
14. **Hoeniger, J. F. M., H. D. Tauschel, and J. L. Stokes.** 1973. The fine structure of *Sphaerotilus natans*. Can. J. Microbiol. **19**: 309–313.
15. **Larkin, J. M.** 1980. Isolation of *Thiothrix* in pure culture and observation of a filamentous epiphyte on *Thiothrix*. Curr. Microbiol. **41**:155–158.
16. **Larkin, J. M., and D. L. Shinabarger.** 1983. Characterization of *Thiothrix nivea*. Int. J. Syst. Bacteriol. **33**:841–846.
17. **Larkin, J. M., and W. R. Strohl.** 1983. *Beggiatoa*, *Thiothrix*, and *Thioploca*. Annu. Rev. Microbiol. **37**:341–367.
18. **Maier, S., and R. G. E. Murray.** 1965. The fine structure of *Thioploca ingrica* and a comparison with *Beggiatoa*. Can J. Microbiol. **11**:645–656.
19. **Merkel, G. J.** 1975. Observations on the attachment of *Thiothrix* to biological surfaces in activated sludge. Water Res. **9**:881–885.
20. **Morita, R. Y., and P. W. Stave.** 1963. Electron micrograph of an ultrathin section of *Beggiatoa*. J. Bacteriol. **85**:940–942.
21. **Nicolson, G. L., and G. L. Schmidt.** 1971. Structure of the *Chromatium* sulfur particle and its protein membrane. J. Bacteriol. **105**:1142–1148.
22. **Ottow, J. C. J.** 1975. Ecology, physiology, and genetics of fimbriae and pili. Annu. Rev. Microbiol. **29**:79–108.
23. **Reichenbach, H.** 1981. Taxonomy of the gliding bacteria. Annu. Rev. Microbiol. **35**:339–364.
24. **Schmidt, G. L., G. L. Nicolson, and M. D. Kamen.** 1971. Composition of the sulfur particle of *Chromatium vinosum* strain D. J. Bacteriol. **105**:1137–1141.
25. **Shively, J. M.** 1974. Inclusion bodies in prokaryotes. Annu. Rev. Microbiol. **28**:167–187.
26. **Snellen, J. E., and H. D. Raj.** 1970. Morphogenesis and fine structure of *Leucothrix mucor* and effects of calcium deficiency. J. Bacteriol. **101**:240–249.
27. **Strohl, W. R., I. Geffers, and J. M. Larkin.** 1981. Structure of the sulfur inclusion envelopes from four beggiatoas. Curr. Microbiol. **6**:75–79.
28. **Strohl, W. R., K. S. Howard, and J. M. Larkin.** 1982. Ultrastructure of *Beggiatoa alba* strain B15LD. J. Gen. Microbiol. **128**:73–84.
29. **Tsien, H. C., and E. L. Schmidt.** 1977. Polarity in the exponential-phase of *Rhizobium japonicum*. Can. J. Microbiol. **23**: 1274–1284.
30. **Williams, T. M., and R. F. Unz.** 1985. Isolation and characterization of filamentous bacteria present in bulking activated sludge. Appl. Microbiol. Biotechnol. **22**:273–282.
31. **Williams, T. M., and R. F. Unz.** 1985. Filamentous sulfur bacteria of activated sludge: characterization of *Thiothrix, Beggiatoa*, and Eikelboom "type 021N" strains. Appl. Environ. Microbiol. **49**:887–898.
32. **Winogradsky, S.** 1888. Beitrage zur Morphologie und Physiologie der Bakterien. I. Zur Morphologie und Physiologie der Schwefelbakterien. Felix, Leipzig 1949. Republished as Contribution à la morphologie et physiologie des sulfobactèries, p. 83–126. *In* S. Winogradsky (ed.), Microbiologie du sol. Masson et Cie., Paris.
33. **Wirsen, C. O., and H. W. Jannasch.** 1978. Physiological and morphological observations on *Thiovulum* sp. J. Bacteriol. **136**: 765–774.