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Microscopic examination and fatty acid characterization of filamentous bacteria colonizing substrata around subtidal hydrothermal vents*

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Abstract. Microscopic examination of the whitish mat that covered the substrata around subtidal hydrothermal vents at White Point in southern California revealed a "*Thiothrix*-like" bacterium containing sulfur inclusions as the dominant filamentous form in this microbial community. The mat-like appearance developed as a result of the closely-packed manner in which the basal ends of the filaments were anchored to the substrate. The dominant phospholipid fatty acids of these filaments (16:0, 16:1w7c, 18:0, 18:1w7c) were similar to those recovered from a sample of *Beggiatoa* isolated from a spring in Florida. Filaments from both sources contained small quantities of C_{18} and C_{20} polyunsaturated fatty acids, as well. A larger but less abundant sheathless, filamentous form, which also contained sulfur inclusions and displayed a cell wall structure similar to a previously described *Thioploca* strain, also colonized the substrata around the subtidal mat. The preservation methods used in the preparation of thin-sections of the subtidal mat material were found to be inadequate for defining some key cellular structures of the large filaments. Nevertheless, the results demonstrate that the filamentous bacteria comprising the microbial mat in the vicinity of the subtidal vents exhibit some of the features of the free-living filamentous microorganisms found in deep-water hydrothermal areas.

Key words: *Thiothrix* sp. – *Beggiatoa* sp. – Sulfide-oxidizing – Polyunsaturated – Fatty acids – Inclusions – Sheath – Southern California – Ultrastructure – Sulfur

A common feature of the various submarine hydrothermal areas discovered to date is a white filamentous bacterial mat which covers the substratum on the sea floor near vents that discharge sulfide-rich hydrothermal fluid into the surrounding seawater (Jannasch 1983, 1985; Stein 1984). Several filamentous bacteria have also been described on the epiderm and on the inner wall of the tube of the polychaete *Alvinella pompejana* which also inhabits the vent area (Desbruyeres et al. 1982).

The mat is comprised of a variety of morphologically-distinct, filamentous bacterial forms, some of which exhibit *Thiothrix*- and *Beggiatoa*-like characteristics (Jannasch 1983 Jannasch and Taylor 1984), and together are likely responsible for a substantial portion of the chemosynthetic production in the vicinity of the vents (Jannasch 1985). Visual observations also suggest that these mats are grazed by a variety of invertebrates.

To date, not much is known about the filamentous microorganisms associated with the mats. This is in part due to the remoteness of the vents which has made sample collection difficult. In addition, attempts to culture these filamentous bacteria in the laboratory have been unsuccessful (Ruby et al. 1981), and efforts to obtain taxonomic information from ultrastructural features in samples of the native population have been compromised by the sensitivity of this group of bacteria to thin-section preparation (Maier and Murray 1965).

Mats of filamentous bacteria have been observed in subtidal areas around hydrothermal vents at White Point in southern California (Stein 1984). These mats are easily accessed and samples may be readily obtained by divers using SCUBA equipment. Specimens can be processed immediately for either enrichment and isolation or fixed for electron microscopic examination. In addition, milligram quantities of mat biomass can be collected for biochemical analyses.

In view of these considerations, we initiated a study to characterize the shallow water mat population. The information obtained from this habitat should lead to a better understanding of the filamentous microflora which populate other less accessible hydrothermal areas in the sea. In this report, we describe some morphological, physiological, and biochemical features of the filamentous bacteria that constitute the mat community found around these shallow coastal vents.

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Materials and methods

Sample collection. White bacterial mats were collected by divers using SCUBA equipment in the vicinity of the subtidal hydrothermal vents in the eastern cove off White Point, California (33° 42′50″ N, 118° 19′00″ W). Filaments comprising the mat were scraped from rock (sandstone) substrate using a scalpel blade and collected in Whirl-Pak plastic sacks as they drifted away from the surface. Filaments attached to the shells of the Norris top snail (*Norrisia norrisii*) and the giant keyhole limpet (*Megathura crenulata*) were collected in a manner similar to those obtained from rock substrates. The filaments were easily disrupted by mechanical agitation so that care had to be taken during sampling in order to recover intact specimens. Filaments attached to macroalgae (*Macrocystis pyrifera*) were sampled in a manner which did not disturb their association with the plant surface. Portions of kelp frond colonized by the white bacterial filaments were excised as 1 cm^2 sections using scissors and forceps and transferred to Whirl-Pak sacks for transport to the surface.

At the surface, the samples were immersed in a buffered fixative solution composed of either 0.067 M cacodylate buffer, pH 8.0, containing 4% NaCl, or 0.28 M Gibb's Veronal-Acetate-Salt buffer, pH 7.5 (Dawes 1971) with 0.5% glutaraldehyde. Ruthenium red (0.015% final concentration) was included in the fixative solution for some preparations.

Light microscopy. Within 48 h of collection, fixed samples were examined directly as wet mounts by phase contrast microscopy.

Epifluorescence microscopy. Fixed samples were transferred to 0.1 M sodium phosphate buffer, pH 8.0, containing acridine orange (10 mg/100 ml buffer, final concentration). After staining for 5 min, portions of the sample were filtered through blackened Nuclepore membranes (0.2 µm pore size) at a vacuum of 15 kg cm^{-2} Hg. The filters were sandwiched between a microscope slide and a glass cover slip with nonfluorescing immersion oil and examined at various magnifications with a Zeiss standard 14 epifluorescence microscope containing a filter combination optimized for detection of acridine orange (Geesey et al. 1978).

Electron microscopy. Within 48 h of collection, the fixed samples were transferred to a buffer solution (0.28 M Gibb's Veronal-Acetate-Salt or 0.067 M cacodylate in 4% NaCl at pH 7.5) containing 5% glutaraldehyde and in some preparations, 0.015% ruthenium red, and gently agitated at 20° C for 1 – 3 h. The samples were washed 5 times in buffer without glutaraldehyde, then post-fixed in buffer solution containing 2% osmium tetroxide and agitated at 20° C for 1 – 3 h. After washing 5 times with buffer to remove unreacted osmium, the samples were either enrobed in 4% agar or directly dehydrated in a graded series of either acetone/water (30, 50, 70, 90, 95, 100%) followed by propylene oxide or the water-soluble polymer, 2-hydroxyethyl methacrylate (GMA) in water (30, 50, 75, 90, 95, 100%). The dehydrated samples were infiltrated first with a 1:1 solution of GMA or propylene oxide and Spurr's resin for 1 h, then with 100% Spurr's resin at 20° C for 2 h. The samples were transferred to fresh resin and polymerized at 70° C for 8 h. Thin-sections were cut on a LKB ultramictrotome, stained with 1% uranyl magnesium acetate and 0.2% lead citrate, and examined with either a Siemens 1 A or an RCA EMU 4 electron microscope.

Sulfur deposition. The deposition of sulfur in filaments recovered from subtidal vents was demonstrated by the method of Skerman as modified by Strohl and Larkin (1978). Filaments were removed from a kelp frond with tweezers and placed on a microscope slide containing a drop of seawater. A drop of pyridine was then added to the slide and the suspension was sealed with a cover slip and petrolatum. Positive results were recorded if the granules disappeared from the filaments and rhombic or monoclinic crystals formed external to the filaments. The reaction was observed by phase contrast and dark field microscopy.

Fatty acid extraction and analysis. The filaments with diameters of around 10 µm were separated from detritus, algal debris, and the large 50 – 75 µm-wide filaments by micromanipulation using disecting needles and a disecting microscope. There were approximately 100 filaments with diameters of 10 µm for every large 50 – 75 µm diameter filament observed during the segregation process. Using this technique it was possible to obtain 200 – 400 mg dry weight material. Following lyophilization, the preparation of narrow filaments was extracted with a modified one-phase chloroform-methanol Bligh and Dyer extraction (White et al. 1979). Phospholipids in the solvent phase were separated on a silicic acid column and the fractions collected and evaporated under a stream of N_2. Fatty acids ester-linked to the phospholipid fraction eluted from the column by methanol were subjected to mild alkaline hydrolysis followed by hexane:chloroform (4:1 v/v) extraction to yield the corresponding fatty acid methyl esters (FAME) (White et al. 1979).

LPS hydroxy fatty acids were recovered by acidification of the sample in 1 N HCl. Samples were then refluxed at 100° C for 3 h, cooled, and extracted in chloroform overnight. The chloroform phase was recovered, the chloroform evaporated under a stream of N_2, and the LPS fatty acids methylated and converted to their corresponding trimethylsilyl (TMSi) ethers with N,O-bis-(trimethylsilyl) trifluoroacetamide (Pierce Chemical Co., Rockford, IL, USA).

Gas chromatography (GC). FAME samples were taken up in hexane with ethylnonadecanoate (19:0) as the internal injection standard. Separation of individual normal and hydroxy fatty acids was performed by high resolution gas chromatography using a Hewlett Packard 5880 A gas chromatograph equipped with a flame ionization detector. Samples were injected at 50° C in a splitless mode with a Hewlett Packard 7672 automatic sampler onto a non-polar cross-linked methyl silicone capillary column (50 m × 0.2 mm i.d., Hewlett Packard). The oven temparature was programed from 50° C to 160° C at 10° C per minute, then at 2° C per minute to 300° C. Hydrogen was used as the carrier gas (1 ml/min). The injector and detector were maintained at 300° C.

Tentative peak identification was based on comparison of retention times with those of commercial and previously identified laboratory standards. Peak areas were quantified with a Hewlett Packard 3350 series programable laboratory data system. Standard deviations for individual fatty acids were generally in the range of 0 – 30%, typically < 10%.

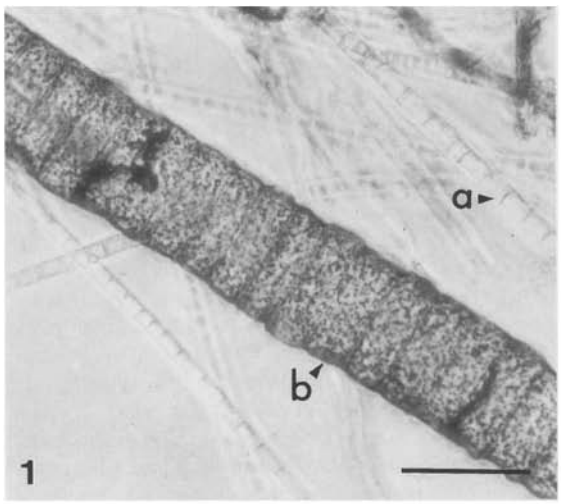
Gas chromatography/mass spectrometry (GC/MS). GC/MS analyses were performed on a Hewlett Packard 5996 A system fitted with a direct capillary inlet. The same column described above was used for analyses. Samples were injected in splitless mode at 100° C with a 30 s venting time, after which the oven temperature was programed to rise to 300° C at 3° C/min. Helium was used as the carrier gas. The electron multiplier was set between 1300 and 1400 V, the transfer line was maintained at 300° C, the source and analyzer were set at 250° C, the electron impact energy was 70 eV, and the system was tuned at m/z 502. Mass spectral data were acquired and processed with a Hewlett Packard RTE-6/VM data system.

Determination of fatty acid double-bond configuration. The dimethyldisufide (DMDS) adducts of monounsaturated FAME were formed by the method described by Dunkelblum et al. (1985) to locate the double-bond postitions. Samples in hexane (50 µl) were treated with 100 µl DMDS and 1–2 drops of iodine solution (6% w:v in diethyl ether) in a standard GC vial fitted with a teflon-lined screw-cap. After reaction at 50° C for 48 h, the mixture was cooled and diluted with 200 µl hexane. Iodine was removed by shaking with 100 µl of 5% aqueous sodium sulfite. The organic layer was recovered and concentrated under a stream of N_2 prior to GC analysis. GC/MS analysis of the DMDS adducts showed major ions attributable to fragmentation between the methyl sulfide groups at the original site of unsaturation. Discrimination between *cis* and *trans* geometry in the original monounsaturated FAME is achieved as a result of the fact that the erythro isomer (originally the *trans* fatty acid) elutes after the threo isomer (originally the *cis* fatty acid). The different positional isomers were separated chromatographically under the conditions used in this study.

Fatty acid nomenclature. Fatty acids are designated by total number of carbon atoms: number of double bonds, followed by the position of the double bond from the methyl end (w) of the molecule. The suffixes c and t indicate *cis* and *trans* geometry and the prefix OH indicates a hydroxyl group at the position indicated.

Results

Mats of filamentous bacteria were observed on a variety of surfaces including rocks, macroalgae, and the shells of invertebrates around subtidal sulfur springs or vents off White Point near Los Angeles, CA. The densely-packed filaments, visible to the naked eye, extended from the substratum 2–20 mm into the surrounding seawater. Examination by phase contrast microscopy of samples from rocks revealed that the mats were composed of a bacterial consortium. Two morphologically-distinct types of filamentous bacteria dominated the microbial community (Fig. 1). Small filaments, 6–9 µm in diameter and 2–10 mm in length, were the most abundant forms present in the mat (Fig. 2). Large filaments, 50–75 µm in diameter and 5–20 mm in length, were much less abundant than the smaller filaments. Their relative abundance appeared to depend to some extent on the type of surface that was sampled. Refractile bodies were observed in both types of filaments (Figs. 1 and 3). Addition of pyridine to wet mounts of the filaments resulted in the complete disappearance of the inclusion bodies and



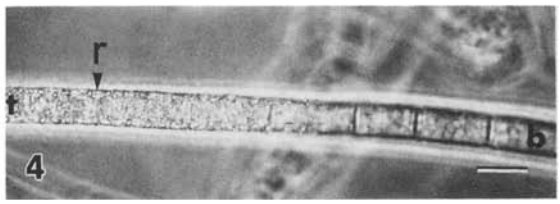


Fig. 1. Phase contrast micrograph of filamentous bacteria recovered from a rock near a subtidal hydrothermal vent. Two morphologically-distinct, filamentous forms (*a* and *b*) dominated the mat community. Bar = 50 µm

Fig. 2. Phase contrast micrograph of filamentous bacteria attached to a kelp frond. Basal end (*e*) anchors the filament to the surface. Tip (*t*) of filament contains more light absorbing material than the basal end. Bar = 25 µm

Fig. 3. Phase contrast micrograph of segments near the tip of several filaments where refractile bodies (*r*) occur in abundance. Bar = 10 µm

Fig. 4. Phase contrast micrograph showing the change in density of refractile bodies (*r*) along the length of a filament between the tip (*t*) and the basal end (*b*). Bar = 10 µm

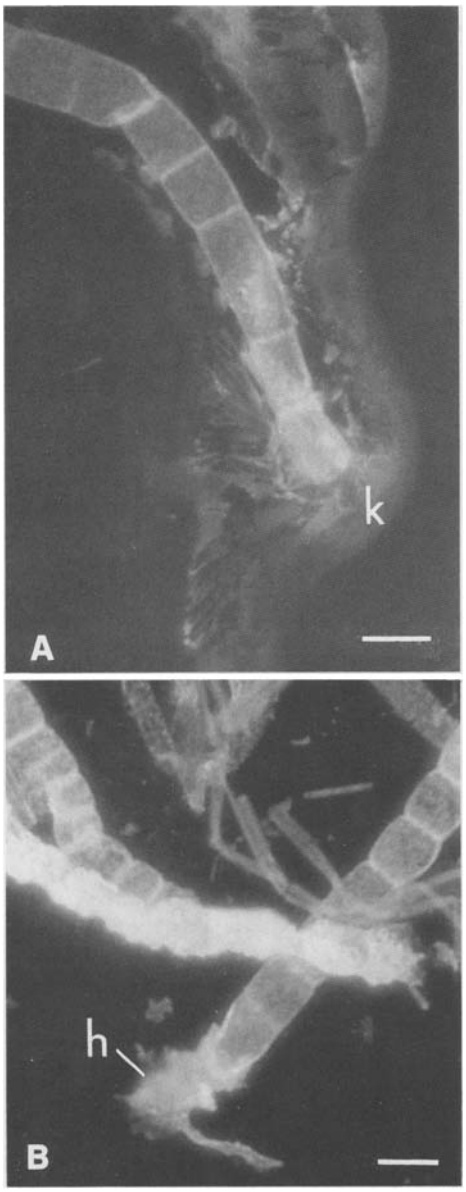


Fig. 5. A Epifluorescent micrograph showing basal end of filament attached to surface of kelp frond (*k*). Note that contact occurs primarily at the terminal cell in the filament. **B** Filament separated from substratum revealing acridine-orange-stained holdfast substance (*h*) extending from filament at polar region of the terminal cell. Bar = 10 μm

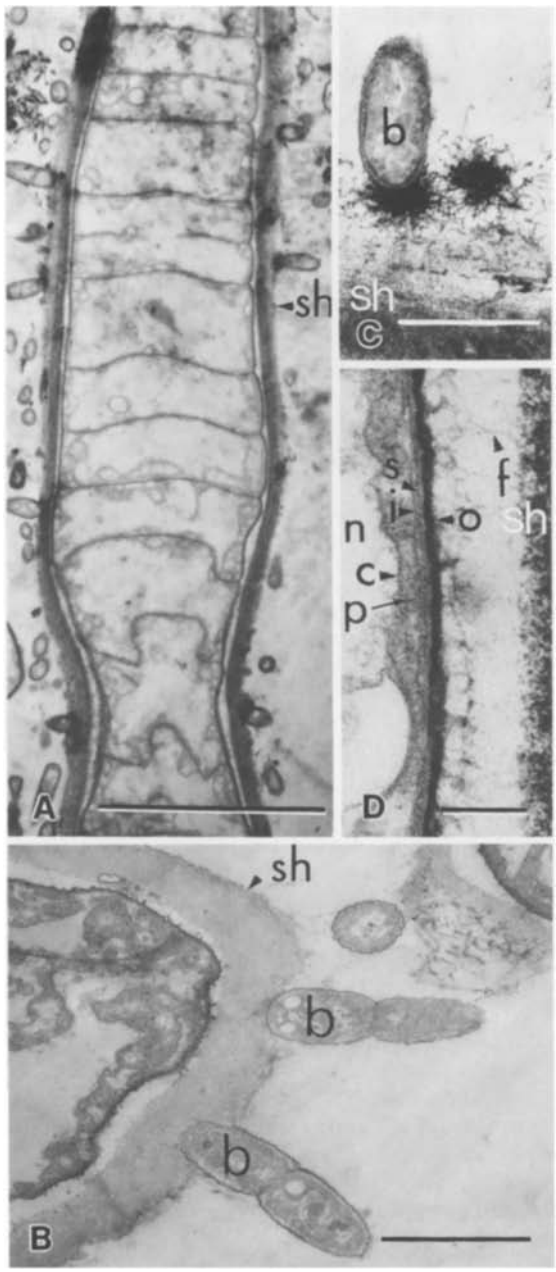


Fig. 6. A Transmission electron micrograph of a longitudinal thin-section of a small filament prepared in the presence of cacodylate-NaCl buffer and ruthenium red. Numerous rod- and coccus-shaped bacteria are associated with the sheath (*sh*) of the filament. Bar = 10 μm. **B** Sample prepared as in **A** showing attachment of polar region of rod-shaped bacteria (*b*) in filament sheath (*sh*). Bar = 1 μm. **C** Enlargement of area in **A** showing condensed fibrous material at point of association between rod-shaped bacteria (*b*) and filament sheath (*sh*). Bar = 1 μm. **D** Enlargement of area of **A** above showing cell wall structure. The sheath (*sh*), is anchored to a dense outer layer (*o*) by fibers (*f*). Two tracks (*s*) and (*i*) appear directly beneath the dense outer layer. An undulating inner layer (*c*) separates the cytoplasm (*n*) from the periplasmic space (*p*). Bar = 0.2 μm

the appearance of crystals, characteristic of sulfur, outside the trichome. The refractile bodies decreased in number from the tip to the basal end of the filament (Fig. 4).

Both large and small filaments were anchored to the substratum at their basal end (Figs. 2 and 5). This orientation promoted the establishment of a densely-packed array of filaments which protruded perpendicular to the substratum. The holdfast was not resolved by either light or electron microscopy, although small filaments associated with fronds of *Macrocystis pyrifera* appeared to be embedded in the gelatinous material coating the surface of the macroalgae when preparations were stained with acridine orange and viewed by epifluorescence microscopy (Fig. 5). Staining with India ink or nigrosin produced a halo around the holdfast material and trichome of the small filaments.

Neither of the filamentous forms occurred in rosettes or produced gonidia when suspended in seawater collected near the vents. Staining with Sudan Black produced no darkened areas within the filaments, indicating the absence of β-hydroxy butyrate inclusions.

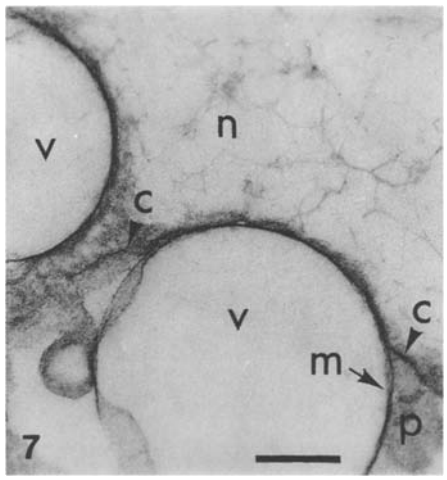


Fig. 7. Electron-transparent vacuoles (*v*) in periplasmic space (*p*) of a cell within the filament shown in Fig. 6A above. Note the juxtaposition of the cytoplasmic membrane (*c*) and the vacuole membrane (*m*). The cytoplasm (*n*) appears to contain filamentous material and electron dense material, possibly ribosomes, in the vicinity of the cytoplasmic membrane. Bar = 0.2 μm

Small filaments

Light microscopic examination demonstrated septa every 10–15 μm along the length of the small filaments, presumably delineating the individual cells (Fig. 4). However, when thin section preparations were examined by transmission electron microscopy the septa were separated by a distance of only 2–3 μm, indicating that the cells were compressed during fixation and dehydration. Filament diameter also appeared to be affected by thin section preparation since it was seen to decrease at points along the length of the filament (Fig. 6A). A thick sheath formed the outermost envelope component of the small filaments. The sheath appeared as a polymeric matrix of varying density, approximately 0.4 μm thick, when samples of mat material were prepared in the presence of ruthenium red and examined as thin sections by transmission electron microscopy (Fig. 6A and B). Distinct layers within the sheath were not observed. Rod- and coccus-shaped bacteria colonized the sheathed filaments (Fig. 6A and B). The rod-shaped bacteria anchored themselves by embedding their polar regions into the sheath of the filament. A condensation of polymeric material was often observed where the rod-shaped bacteria contacted the sheath of the filamentous bacteria (Fig. 6C).

The small filaments possessed an envelope composed of several distinguishable layers (Fig. 6D). Beneath the sheath was a thick, electron-dense layer (o). The outer surface of the o layer contained fibrous material which extended to the sheath. Two dark tracks (s and i), separated by a light area, occurred immediately beneath the o layer. A highly convoluted layer (c), presumably the cytoplasmic membrane, was separated from the other envelope layers by an extensive periplasmic space (p). Membrane-enclosed, electron-transparent vacuoles were located within the periplasmic space. In some cross-sections, the vacuole membrane appeared to converge with the cytoplasmic membrane (Fig. 7). To date, we have been unable to determine which cell wall layers are continous with the septa.

The small filaments yielded a phospholipid fatty acid (PLFA) profile that was very similar to that obtained from a sample collected from a spring in Newport, Florida (30° 11′80″ N, 84° 10′90″ W) known to contain *Beggiatoa* (Fig. 8) and to a PLFA profile from bacteria recovered recently from deep-sea hydrothermal vents (data not shown). The filamentous bacteria from the subtidal vents and the Florida spring shared as their dominant PLFA 16:1w7c, 16:0, and 18:1w7c. The only other major PLFA recovered from the subtidal filaments that was not abundant in the filaments from the Florida spring was 18:0. The fatty acids 15:0, 15:1, 17:0, and 17:1, which were present in small amounts in the *Beggiatoa* samples from the Florida spring, were not detected in the small subtidal filaments. The filaments from both sites contained small amounts of C_{18} and C_{20} polyunsaturated fatty acids (PUFA) which are rarely found in procaryotes.

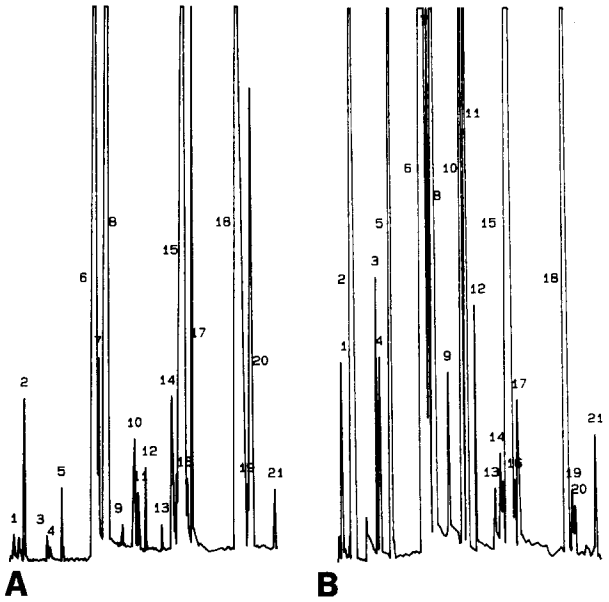


Fig. 8A, B. Phospholipid fatty acid profiles of **A** preparation of 10 μm diameter filaments from subtidal vents and **B** preparation of *Beggiatoa* from Florida sulfur spring. Peak identification was as follows: *1* (14:1), *2* (14:0), *3* (15:1w8c), *4* (15:1w6c), *5* (15:0), *6* (16:1w7c), *7* (16:1w5c), *7a* (16:1w13t), *8* (16:0), *9* (unidentified), *10* (17:1w8c), *11* (17:1w6c), *12* (17:0), *13* (C_{18} PUFA), *14* (C_{18} PUFA), *15* (18:1w7c), *16* (18:1w5c), *17* (18:0), *18* (19:0 internal standard), *19* (20:4w6), *20* (20:5w3), *21* (20:1)

The LPS fraction recovered from the small subtidal filaments also contained the same hydroxy fatty acid as that recovered from the *Beggiatoa* sample obtained from the Florida springs. 2-OH 14:0, which contributed >90% of the hydroxyl fatty acids in the LPS fraction of the *Beggiatoa* sample, occurred as the sole hydroxy fatty acid in the LPS fraction of the filaments from the subtidal vents.

Large diameter filaments

Individual cells within each large filament were 15–20 μm in width and 50–75 μm in length. The envelope of the large filaments exhibited a structure which was distinct from that of the narrower filaments described above. Three discrete layers, a lightly-stained, broad, inner layer (i) and 2 thinner, electron-dense, outer layers (x and y) of similar width were resolved by transmission electron microscopy (Fig. 9A). We have not yet identified the relationship between the septum and the filament envelope.

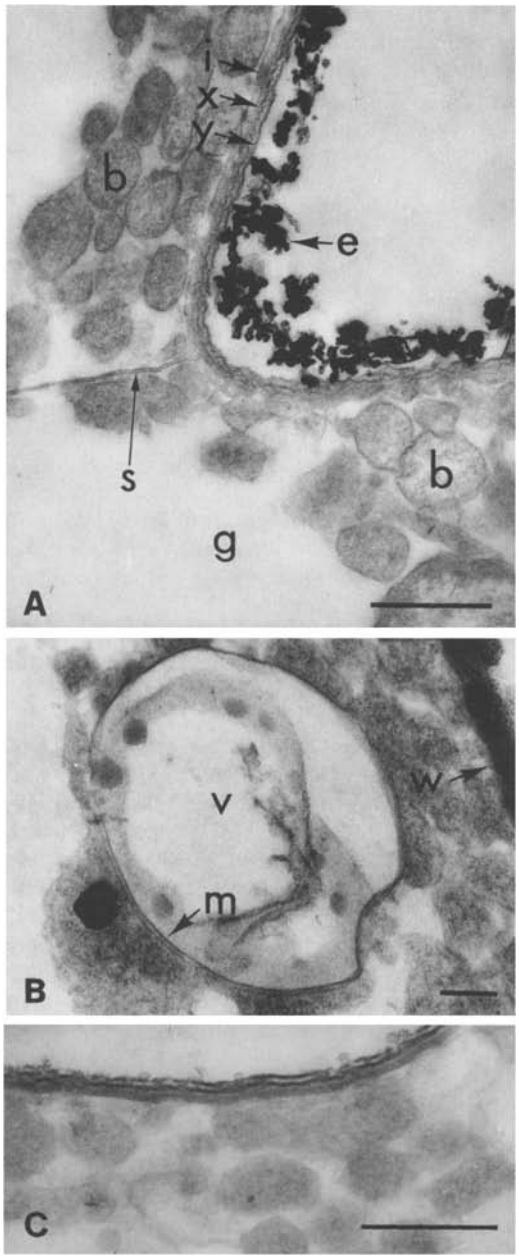


Fig. 9. A Transmission electron micrograph of a thin-section of a large filament prepared in the presence of Gibb's buffer and ruthenium red and enrobed in 4% agar. Electron dense bodies (*b*) are concentrated along the septum (*s*) and lateral wall. The wall is composed of a broad, lightly-stained inner layer (*i*) and 2 electron-dense, outer layers (*x* and *y*). Condensed, electron-dense material (*e*) appears on the outer surface of the filament. The central area of the cell (*g*) appears void of cytoplasmic constituents. Bar = 0.5 μm. **B** Electron-transparent vacuole (*v*) enclosed by a membrane (*m*) near the lateral wall (*w*) of the cell in **A** above. Bar = 0.2 μm. **C** Transmission electron micrograph of a thin-section of a large filament prepared in a manner similar to that in **A** above but without ruthenium red. Note the absence of the condensed, electron-dense material at the outer filament surface in this preparation. Bar = 0.5 μm

Electron-dense bodies as well as membrane-enclosed, electron-transparent vacuoles were seen clustered at the periphery of the inner surface of the septum and lateral wall (Fig. 9A and B). Although the perimeter of the electron-dense bodies appeared darker than the internal material, no membrane structure was evident. We have not yet identified the contents of these bodies. Furthermore, since we have not yet resolved the cytoplasmic membrane in thin-section preparations of the large filaments, it is not known whether the bodies and vacuoles lie within the cytoplasm or periplasmic space. The central area of each cell within the large filaments was electron transparent and appeared void of cytoplasmic constituents. We have not observed a membrane separating this void area from the area containing the electron-dense bodies and electron-transparent vacuoles.

Deposits of condensed, electron-dense material (e) were observed at the outer surface of the large filaments (Fig. 9A). This condensed material was absent in preparations that did not include ruthenium red (Fig. 9C).

Discussion

Visual observations made during SCUBA dives confirmed previous reports of a conspicuous white bacterial mat which covered the substratum in the immediate vicinity of subtidal hydrothermal vents at White Point in southern California (Stein 1984). The large and small filamentous bacteria, which formed the mats, exhibited features which are characteristic of sulfur-oxidizing bacteria. The disappearance of the numerous refractile bodies from the filaments and the appearance of crystals, characteristic of sulfur, outside the trichome during reaction with pyridine suggests that the numerous vacuoles seen by electron microscopy were sites of sulfur deposition. Since there was an abundance of hydrogen sulfide in the fluid discharged from the springs (Stein 1984), it is likely that both large and small subtidal filaments oxidize hydrogen sulfide to elemental sulfur. That the number of refractile bodies decreased from the apical tip to the basal end of the filaments indicates that cells in different segments of a single filament are physiologically-distinct.

The existence of a unit membrane around the electron-transparent vacuoles in both large and small subtidal filaments is a common feature of sulfur inclusions in *Thiothrix*, *Thioploca*, and *Beggiatoa* (Maier and Murray 1965; Strohl et al. 1981; Larkin and Strohl 1983; Williams et al. 1987). Since the electron-dense bodies in the large filaments did not appear to be surrounded by a membrane, they likely contain products other than sulfur. Like the vacuoles in the 3 genera of filamentous sulfur-oxidizing bacteria mentioned above, those in the small subtidal filaments appeared to be located outside the cytoplasmic membrane. The vacuoles in the large subtidal filaments may also maintain a periplasmic location, but our inability to delineate the cytoplasmic membrane leaves this point unresolved.

The attachment of individual large and small subtidal filaments to the substratum via their basal end is characteristic of *Thiothrix*. This orientation permitted maximum utilization of available space by these sessile microorganisms and promoted the carpet-like coverage on exposed surfaces surrounding the vents.

The rosettes structures and gonidia, commonly observed in samples containing *Thiothrix*, were not observed in our preparations of large and small filaments. Larkin (1980) reported, however, that under some laboratory culture conditions, rosettes were not formed by *Thiothrix* isolates. Bland and Staley (1978) were unable to detect gonidia in samples of *Thiothrix* collected from freshwater springs. Thus, our inability to observe these structures in our samples does not

necessarily exclude the large and small subtidal filaments from the genus *Thiothrix*.

Although the large and small subtidal filaments shared several important morphological and physiological features, differences were noted which suggests they are not the same species. The diameter of the large and small filaments differed by a factor of 5–7. While filament diameter is known to vary among otherwise similar filaments within a sample (Jannasch and Wirsen 1981), the magnitude of the variation has never approached that observed between the large and small subtidal filaments described here. Except for the *Beggiatoa*-like microorganisms in the soft sediment around the Guaymas Basin vent site in the Gulf of California, which exhibit diameters of up to 160 μm (Jannasch 1983), the 50–75 μm diameter subtidal filaments are larger than any other sulfur-oxidizing bacteria described to date.

In addition to filament diameter, the large and small subtidal filaments differed in their cell wall architecture, even when the same fixation and staining procedures were employed. The wall structure of both types of filaments was more complex than that of a typical Gram-negative bacterium. The walls of both displayed the extra layers often found in the genera *Thiothrix*, *Thioploca* and *Beggiatoa*. The 3-layer wall structure of the large filaments resembled the envelope of a *Thioploca* reported by Maier and Murray (1965). However, *Thioploca* filaments typically exist in bundles surrounded by a common sheath, a characteristic which was not evident in the large subtidal filaments. The absence of a definable sheath around the large filaments is also inconsistent with the characteristics of *Thiothrix*, although Williams and Unz (1985) have observed strains which lack a sheath. It is possible that the poorly preserved material detected on the outer surface of individual large filaments was the remnant of a sheath or extra layer. That this material is an artifact of sample preparation is unlikely since it was not observed in otherwise identical preparations that lacked ruthenium red. Nevertheless, a more effective fixation and staining procedure will have to be employed for preparation of the large filaments for transmission electron microscopy before the complete envelope structure is elucidated.

The small filaments exhibited a cell wall structure that was most similar to that of *Thiothrix*. The envelope of the small subtidal filaments differed, however, from the wall structure of *Thiothrix* depicted by Larkin and Strohl (1983) and that of Strain Q described by Williams et al. (1987) in that it possessed an additional electron dense layer immediately beneath the sheath. This layer may, however, be a part of the sheath that separated from the bulk of the sheath material during fixation and thin-section preparation. The sheath of some *Thiothrix* spp. appear as several separate layers (Williams et al. 1987).

Epiphitic bacterial populations such as those associated with the small subtidal filaments have also been reported in *Thiothrix* recovered from sulfide-containing freshwater springs (Morita and Burton 1965; Larkin 1980). Whereas the epiphytes on the subtidal filaments were rod-shaped bacteria, the epiphytes on the freshwater *Thiothrix* strains were themselves filamentous bacteria which extended perpendicular to the host filament. The rod-shaped epiphytes do not appear to be gonidia that reportedly attach to the filaments via polar fimbriae (Larkin and Strohl 1983). Our study shows the polar region of the rod-shaped bacteria embedded in the sheath of the host filament. The difficulty in obtaining pure cultures of *Thiothrix* may therefore arise from the intimate physical relationship that these microorganisms maintain with other bacteria.

The detection of PLFAs and OH-fatty acids in lipid extracts of the small filaments indicates that they are eubacteria. The small quantities of epiphytic bacteria that were associated with the filaments could not have accounted for the quantities of fatty acids that were recovered from the sample. The similarity of the PLFA profile of the small subtidal filaments to that obtained from the sample containing *Beggiatoa* sp. indicates that the envelopes of the 2 filamentous forms are closely related, biochemically. Unfortunately, the significance of this comparison cannot be fully appreciated since PLFA profiles of *Thiothrix* and *Thioploca* spp. are not available at this time. The recovery of 2-OH 14:0 as the sole 2-OH fatty acid suggests that the small subtidal filaments are not closely related to the *Flexibacter*, *Vitreoscilla* or *Filibacter* species examined to date which have 2-OH 12:0 as their major hydroxy fatty acid (Nichols et al. 1986). Significant differences also appeared between the PLFA profiles of these gliding bacteria and that obtained from the subtidal filaments.

The detection of C_{18} and C_{20} PUFAs in preparations of the subtidal filaments, the deep vent bacteria, and the *Beggiatoa* samples from the Florida springs was quite unexpected. Although these fatty acids are typical in eucaryotic algae and cyanobacteria, they have only been reported in a few eubacteria. The gliding bacterium, *Flexibacter polymorphus* was reported to contain a C_{20} PUFA (Johns and Perry 1977), and more recently, C_{20} and C_{22} PUFAs were detected in a number of deep-sea bacterial isolates and in *Vibrio marinus* (DeLong and Yayanos 1986). Since neither the preparation of small filaments from the subtidal vent or the *Beggiatoa* from the Florida spring were derived from pure cultures, we cannot rule out the possibility at this time that the PUFAs were contributed by algal contaminants. In view of these findings, it would be interesting to determine whether PUFAs exist in the filamentous bacteria around the deep hydrothermal vents where photosynthetic organisms are conspicuously absent.

In summary, the accessibility of the shallow marine sulfur vents at White Point provided the opportunity to collect sufficient quantities of free-living filamentous microorganisms to test various methods of fixation and staining for preservation of filament structure and for biochemical analysis. The small filaments which dominated the mat community exhibited features which most closely resembled those of the genus *Thiothrix*, while the larger, less abundant filaments displayed the sessile existence of *Thiothrix* and an envelope structure like *Thioploca*. The results of this study should serve as a useful basis for comparison when data on the microbiology of the filamentous bacteria in the more remote deep-water hydrothermal vents becomes available.

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