Characterization of Large, Autotrophic *Beggiatoa* spp. Abundant at Hydrothermal Vents of the Guaymas Basin†

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Filamentous bacteria, identified as members of the genus *Beggiatoa* by gliding motility and internal globules of elemental sulfur, occur in massive aggregations at the deep-sea hydrothermal vents of the Guaymas Basin, Gulf of California. Cell aggregates covering the surface of sulfide-emanating sediments and rock chimneys were collected by DS R/V *Alvin* and subjected to shipboard and laboratory experiments. Each sample collected contained one to three discrete width classes of this organism usually accompanied by a small number of “flexibacteria” (width, 1.5 to 4 μm). The average widths of the *Beggiatoa* classes were 24 to 32, 40 to 42, and 116 to 122 μm. As indicated by electron microscopy and cell volume/protein ratios, the dominant bacteria are hollow cells, i.e., a thin layer of cytoplasm surrounding a large central liquid vacuole. Activities of Calvin-cycle enzymes indicated that at least two of the classes collected possessed autotrophic potential. Judging from temperature dependence of enzyme activities and whole-cell CO₂ incorporation, the widest cells were mesophiles. The narrowest *Beggiatoa* sp. was either moderately thermophilic or mesophilic with unusually thermotolerant enzymes. This was consistent with its occurrence on the flanks of hot smoker chimneys with highly variable exit temperatures. In situ CO₂ fixation rates, sulfide stimulation of incorporation, and autoradiographic studies suggest that these *Beggiatoa* spp. contribute significantly as lithoautotrophic primary producers to the Guaymas Basin vent ecosystems.

Dense animal communities at most deep-sea hydrothermal vents are largely, if not entirely, dependent on the production of organic carbon by lithoautotrophic bacteria. In the presence of geothermally generated hydrogen sulfide as the electron donor and free oxygen of the ambient seawater, this life-supporting interaction in a permanently dark environment occurs predominantly in endosymbiotic associations (5, 10). A significant part, however, is contributed by free-living bacteria emitted in the plumes of the warm (ca. 20°C) hydrothermal fluid and in dense microbial mats as food for filter-feeding and surface-grazing animals. In addition to symbiotic and free-living sulfur- as well as methane-oxidizing bacteria, free-living types also include extremely thermophilic anaerobic lithoautotrophs (methanogens) and sulfur-reducing heterotrophs (13, 14).

In certain locations, the most conspicuous microbial mats consist of large filamentous sulfur bacteria of the genera *Beggiatoa* and *Thiothrix* (11, 12). Both of these genera have long been believed to contain strains capable of chemoautotrophic growth at the expense of sulfide oxidation in a medium devoid of organic compounds (17, 38). However, amidst numerous studies in which *Beggiatoa* strains did not show evidence of chemoautotrophic growth (summarized in reference 19), there have been only a few confirmations of chemoautotrophy in pure culture for *Beggiatoa* spp. (25, 26).

Unusually heavy mats of *Beggiatoa*-like filaments were observed when the Guaymas Basin vents were discovered in the Gulf of California (P. F. Lonsdale, Abstr. EOS, Am. Geophys. Union 61:995, 1980). This particular deep-sea vent site (2,000 ± 10 m) differs from all other known tectonic spreading centers by the fact that the emission of the 350°C hydrothermal fluid from the lava bedrock occurs below and passes through a 400-m layer of relatively rapidly depositing sediment (1 m/1,000 years). These deposits represent a mixture of terrigenous and pelagic material, the latter adding a significant organic component of largely diatomic origin (32). The hydrothermal fluid reaches the sediment surface in typical hot smokers with exit temperatures of 130 to 355°C or, in more peripheral areas of sediment, as slow pore-water extrusions. The latter commonly exit at temperatures below 100°C and are quickly cooled by the 3°C bottom seawater, but pore-water temperatures can increase up to about 180°C at a sediment depth of 80 cm (H. W. Jannasch, in D. L. Wise, ed., Bioprocessing and Biotreatment of Coal, in press).

After initially studying a preserved sample of a bacterial mat from this site (9), we had the opportunity to participate in follow-up cruises during the summer of 1985 and the winter of 1988. *Beggiatoa* filaments were observed loosely attached or floating directly above H₂S-emanating sediments. These mats were one to several centimeters thick and occurred in patches several meters across interspaced by areas of black sediment. Massive mats were also observed within stands of vestimentiferan tube worms (*Riftia* sp.) on the nearly vertical sides of large hot smoker vent chimneys. At the center of these mats, the aggregations were up to 60 cm thick and engulfed the tube worms up to their red gill plumes. The bacteria were collected with a vacuum cleaner-like device, dubbed a slurp gun. Although the organisms were not successfully cultured, we were able to conduct a number of studies that are described in this report.

**MATERIALS AND METHODS**

**Samples.** Samples were collected during *Atlantis II/Alvin* cruise no. 112/28 (dives 1603 to 1616; July to August 1985) and cruise no. 118/28 (dives 1965 to 1970; January to February 1988) at the Guaymas Basin vent site, southern section (27°01' N, 111°24' W; depth, 2,004 m) with two types
of slurp guns (orifices 2 and 8 cm wide and collecting jars of 2- and 18-liters volume, respectively). A minimum of 2 h elapsed between collection and arrival of the material aboard ship, during which the samples were exposed to temperatures ranging from 3°C (ambient bottom seawater) to 25°C (surface water). Samples were processed immediately after collection or stored at 4°C for up to 24 h. During the holding period, dense mats were reestablished in the collecting jars.

Alvin dives 1615 and 1970 resulted in the largest samples from the hot smoker chimney walls (see above). Portions of approximately 10 to 50 g (wet weight) were centrifuged (12,000 × g, 20 min) and stored at −80°C until used for enzyme assays. These bulk collections are represented by filament suspensions 1615B and 1970 (Table 1). Smaller collections from sediment surfaces (dives 1606, 1966, and 1968) or chimney walls (dive 1612) were also frozen.

Preparation of filament suspensions. On board ship, filaments from the slurp gun samples were concentrated by centrifugation (4,000 × g, 2 min) or settling at room temperature and were suspended in either filtered (0.2-μm pore size) or autoclaved natural seawater at a density of 0.1 to 3.2 mg of protein per ml. Suspensions are designated by dive number followed by a letter if the sample was subdivided and variously treated. Some suspensions were cleaned by one of several methods (Table 1) in an attempt to remove remaining unicellular bacteria.

Determination of filament volume and width. A sample (0.1 to 2.0 ml) of each filament suspension was immediately diluted 10- to 100-fold into 2.5% glutaraldehyde-supplemented sterile natural seawater or phosphate buffer (0.1 mM, pH 7.0) and stored at 4°C until analysis. In the laboratory, appropriate dilutions were stained with acridine orange, evenly distributed by gentle suction onto black Nuclepore filters, and viewed by epifluorescence microscopy (8). Several discrete width classes of filamentous bacteria were clearly recognizable for any single preparation. For each width class, total filament length was estimated by counting vertical and horizontal intersections of filaments with a superimposed grid (30). For each width class of each sample, a minimum of 400 intersections were counted and the diameters of at least 40 filaments from each width class were measured. In addition to shipboard measurements of live material, wet mount and filtered preparations of preserved material were used to measure the effect of preservation and filtration on filament width. In live material of strain 81-6, the average filament width was approximately 1.2 times that of preserved cells. This ratio ranged from 0.87 to 1.25 for five comparisons of average width (n = 10 to 40) for vent *Beggiatoa* spp. of dives 1966, 1968, and 1970. The biolume of filaments of each width class was calculated from average length and width values assuming a cylindrical shape (diameter = width).

**Table 1.** Width classes of *Beggiatoa* spp. and flexibacteria-like filaments and their respective contributions to total cell volume in filament suspensions derived from samples of various dives

<table>
<thead>
<tr>
<th>Suspension</th>
<th>Wide <em>Beggiatoa</em></th>
<th>Medium <em>Beggiatoa</em></th>
<th>Narrow <em>Beggiatoa</em></th>
<th>Flexibacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg width (µm)</td>
<td>Cell avg width (%)</td>
<td>Avg width (µm)</td>
<td>Cell avg width (%)</td>
</tr>
<tr>
<td>1606A</td>
<td>122</td>
<td>0.21</td>
<td>42</td>
<td>1.6</td>
</tr>
<tr>
<td>1606B</td>
<td>116</td>
<td>0.17</td>
<td>40</td>
<td>2.0</td>
</tr>
<tr>
<td>1612A</td>
<td>28</td>
<td>0.36</td>
<td>2.2</td>
<td>0.0012</td>
</tr>
<tr>
<td>1612B</td>
<td>29</td>
<td>0.36</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td>1612C</td>
<td>27</td>
<td>0.77</td>
<td>0.0023</td>
<td></td>
</tr>
<tr>
<td>1615A</td>
<td>31</td>
<td>0.34</td>
<td>0.0013</td>
<td></td>
</tr>
<tr>
<td>1615B</td>
<td>30</td>
<td>0.17</td>
<td>0.0044</td>
<td></td>
</tr>
<tr>
<td>1966</td>
<td>15</td>
<td>ND</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>118</td>
<td>18</td>
<td>0.008</td>
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</tr>
<tr>
<td>1970</td>
<td>32</td>
<td>0.31</td>
<td>0.013</td>
<td></td>
</tr>
</tbody>
</table>

* ND, Not determined.

* Cell suspension designation is Alvin dive number, followed by a letter if sample was subdivided and subjected to different treatments.
* Cell volume expressed as a percentage of suspension volume, i.e., (cubic centimeters of cell volume per milliliter of suspension) × 100%.
* Filament suspension (4,000 × g, 2 min) followed by suspension in 15 ml of sterile natural seawater plus 200 µM sulfide (SNSW + S). Repeated three times.
* Cleaned by retention on 64-µm mesh while washing with SNSW + S. Repeated three times.
* Cleaned by allowing dispersed filaments to clump, resuspension in 25 ml of SNSW + S, and centrifugation (4,000 × g, 2 min).

**Determination of total protein.** Samples (0.5 to 5.0 ml) of each filament suspension were centrifuged (12,000 × g, 20 min), and the cell pellets were immediately frozen (−80°C). Samples were transported on dry ice and stored at −80°C. Before assay, the cell pellets were thawed, suspended in assay buffer (100 mM Tris hydrochloride, 20 mM MgCl₂, 5 mM NaHCO₃, 6.5 mM dithiothreitol, pH 8.2), passed twice through a chilled French pressure cell (1.1 × 10⁵ kPa), and centrifuged (12,000 × g, 10 min, 4°C) to remove debris. The resultant cell extract was assayed for the activities of d-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO; EC 4.1.1.39) and ATP: d-ribulose-5-phosphate-1-transferase (phosphoribulokinase, EC 5.3.1.6) according to procedures detailed elsewhere (28), with the exception that exogenous RuBisCO was not added to the phosphoribulokinase assays.

**Carbon dioxide fixation.** Shipboard experiments were conducted to measure the ¹⁴CO₂ fixation capacity of a freshly collected filament suspension, sometimes after cleaning (Table 1). Portions of the suspension were supplemented with Na₂S (100 to 500 µM) or Na₂S,SO₃ (10 mM) or left unsupplemented (control). These were then incubated at temperatures ranging from 8 to 45°C under a normal (20%) O₂ gas phase unless a reduced O₂ gas phase (0.8%, balanced by N₂) is indicated. Subsamples were removed at 0.5- to 1-h intervals over a 3-h period, filtered, washed, and fumed over HCl (37). The amount of carbon fixed into cell material was calculated from data obtained with a Beckman LS-100 liquid scintillation counter. Incorporation data are expressed relative to dry weight, which was measured directly (less the tare weight of an average filter) for dense suspensions or computed as twice the protein value of more dilute suspensions.

A Plexiglas sampling-incubating device was used by the research subsurface during several dives for in situ measurements of CO₂ fixation. Filament tufts were collected by pushing the sampler slowly into a thick mat of *Beggiatoa* spp. and closing it before injecting NaH¹⁴CO₃ from an attached syringe. Each sample was incubated in situ (3°C and 200 atm) for 5.2 to 7.2 h before it was retrieved by the subsurface and returned to the ship for analysis by the procedure described above.
Electron microscopy and autoradiography. Samples fixed in phosphate-buffered glutaraldehyde (2.5%) immediately after retrieval were prepared for transmission electron microscopy by published methods (12). They were dehydrated in a graded series of ethanol followed by two 5-min exchanges in propylene oxide. The samples were embedded in Spurr epoxy resin and treated with Reynolds stain (2% aqueous uranyl acetate and lead citrate). Thin sections were examined and photographed with a Zeiss 10 CA electron microscope. Autoradiography was conducted in parallel to the CO₂ fixation experiments with filament suspension 1966. The filaments were incubated in sterile artificial seawater with 100 μM sulfide and 5.0 μCi of NaH¹⁴CO₃, per ml at both 19 and 48°C. Filtering, coating, and exposure of Kodak NTP-2 emulsion were done by the method of Tabor and Neihof (36).

Miscellaneous methods. Duplicate samples from suspensions 1606A and 1615B were filtered (type AA, 0.45-μm pore size; Millipore Corp., Bedford, Mass.) and stored frozen until assayed for elemental sulfur (S⁰). The filters were dried (60°C, 24 h) and then twice extracted with spectral-grade CS₂. After the solvent was transferred and evaporated, the dried residue was dissolved in petroleum ether (90 120°C boiling fraction). After cyanalysis, S⁰ was assayed spectrophotometrically (1). The S⁰ contributed by the solvent and filters was less than 5% of the total.

As a source of RuBisCO from a mesophilic Beggiatoa sp., a chemoautotrophic strain (MS-81-6) was grown at 25°C in sulfide-oxygen gradient medium (8 mM sulfide agar) as detailed by Nelson and Jannasch (25).

RESULTS

Microscopic observations of preserved material. When measurements of filament widths in all suspensions were compared (Table 1), three Beggiatoa groups emerged (Fig. 1). The distinct clusters indicate discrete groupings rather than a continuum of widths. Many of the larger Beggiatoa-type filaments exceeded 1 cm in length. If a suspension contained only a single width class of Beggiatoa spp. (dives 1612, 1615, and 1970), it was always the narrow one. Three widths were present in suspensions from dive 1606. Narrow and wide Beggiatoa spp. were present in suspensions 1966 and 1968, and each was similar in average width to the corresponding class in other suspensions (dives 1606, 1612, 1615, and 1970). The narrow Beggiatoa filaments of suspensions 1606A and 1606B differed statistically in width from the narrow ones of the other suspensions, but this may be a result of differences in preservation methods (see below).

All classes of filaments except the narrowest (the "flexibacteria") were judged to be members of the genus Beggiatoa by the following criteria.

(i) They contained numerous internal S⁰ globules, which are deposited by Beggiatoa spp. in the presence of sulfide or thiosulfate (24, 35). Suspensions 1606A and 1615B contained S⁰ at 8 and 18% (weight of S⁰ per weight of total protein), respectively. An independent determination (R. Steudel, personal communication) from freeze-dried material of suspension 1615B resulted in a total sulfur content of 14% (wt/wt, determined by oxidation with Br₂ followed by titration of the sulfate with BaCl₂) and 13.6% elemental sulfur (extracted with CS₂ and identified by high-pressure liquid chromatographic analysis) largely present as S₂ with only traces of S₇. As in earlier studies on sulfur globules from Chromatium species, no polythionates were found (34).
(ii) They were capable of gliding motility. The narrow filaments (dives 1612, 1615, and 1970) and the medium-width filaments (dive 1606) showed speeds of 3 to 6 and 2 to 3 μm/s, respectively, at 25°C. These rates are typical of narrow Beggiatoa strains (23). Preliminary studies with material from dive 1606 suggested that soluble sulfide (100 μM, pH 8.4) stimulated the rate of gliding of the medium-width Beggiatoa sp. The gliding rate of the widest filaments (dives 1606 and 1966, Table 1) was 1 to 2 μm/s.

Beggiatoa spp. are classified as colorless bacteria because pure cultures have no obvious coloration apart from the pink imparted to dense suspensions by cytochromes. Based on material from suspension 1615B, Prince et al. (31) demonstrated that an unusually high content of c-type cytochromes
is responsible for the bright pink color of these bacteria. Dithionite-reduced minus air-oxidized spectra of suspensions 1968 and 1970 indicated an equally high content of c-type cytochromes (D. C. Nelson, unpublished data).

The suspensions from all dives except dive 1606 also contained thin filamentous bacteria (Table 1; average width, 2.0 to 2.6 μm; cell length, 6 to 10 μm) which are designated here as flexibacteria. No evidence of S⁰ globules was detected in these organisms by microscopic examination. Vibrations on shipboard made it difficult to determine whether these filaments possessed the relatively slow gliding and flexing motility characteristic of flexibacteria. They contributed less than 0.5% to the total biovolume of filamentous bacteria in every suspension except 1970 (Table 1). There is no evidence that various cleaning procedures employed altered the biovolume ratio of flexibacteria to Beggiatoa spp.

CO₂ fixation. During the 1985 expedition, only one of four experiments (suspension 1612A) showed a significant shipboard uptake of ¹⁴CO₂ (Fig. 2A). At 28°C, there was a slight stimulatory effect from the inclusion of 100 μM sulfide, and the maximal rate of incorporation observed was 0.36 nmol of CO₂ · mg (dry weight)⁻¹ · min⁻¹. The rate of incorporation at 45°C was more than 1 order of magnitude lower and not sustained beyond 1 h. In this experiment, the cleaning of the filament suspension was minimal, consisting of pipetting into a separate vessel, settling for a few minutes, and repipetting. Nonetheless, the rate of fixation began to decline after 1 h and significant cell lysis commenced after 2 h. More vigorous cleaning procedures employed with other samples, especially centrifugation, appeared to stop fixation entirely (data not shown) despite the reduced oxygen tensions employed in some experiments.

During the 1988 expedition, suspensions were subjected to minimal manipulation before experiments and all showed significant CO₂ incorporation. For suspension 1970, 500 μM sulfide-stimulated CO₂ fixation almost sevenfold relative to un-supplemented controls (Fig. 2B). At 8°C, this sulfide-stimulated incorporation increased in an approximately linear fashion (0.02 nmol of CO₂ · mg of protein⁻¹ · min⁻¹) for

![Figure 2](http://aem.asm.org/)

**FIG. 2.** Incorporation of ¹⁴CO₂ from bicarbonate by Beggiatoa spp. (A) Control 1612A, single width class of Beggiatoa sp. (ξ = 28 μm). Symbols: ■, 45°C; 100 μM sulfide; ●, 28°C; 100 μM sulfide; ○, 28°C, control (no additions); (B) suspension 1970, 8°C, single width class of Beggiatoa sp. (ξ = 32 μm). Symbols: ●, 500 μM sulfide; ○, 100 μM sulfide; △, control.

![Figure 3](http://aem.asm.org/)

**FIG. 3.** Incorporation of ¹⁴C from bicarbonate by various shipboard suspensions of Beggiatoa spp. and one in situ incubation. Rates are maximum values sustained for 1 to 3 h. Symbols: — — — — suspension 1970; ●, 500 μM sulfide; ○, 100 μM sulfide; ○, control; — — — — suspension 1968; ■, 500 μM sulfide; △, 10 mM thiosulfate; □, control; △, suspension 1966; ▲, 300 μM sulfide; △, 100 μM sulfide; control (not shown) equivalent to 100 μM sulfide; — — — — suspension 1612; ●, 100 μM sulfide; ○, control; △, dive 1970, in situ rate.
at least 3 h. The same general pattern was observed at 19°C, but the maximum fixation rate was roughly four times that measured at 8°C. These and other results of CO₂ incorporation experiments are summarized in Fig. 3. Thiosulfate did not stimulate incorporation beyond the control level in any experiment. For experiments in which the maximum rate of incorporation was achieved in the absence of sulfur supplements, internal stores (5⁹ or organic polymers) are postulated to have provided the necessary energy source.

The results of one in situ CO₂ incorporation experiment (dive 1970) were similar to those obtained in shipboard measurements. In this sample of very clean filaments, a rate of 0.12 nmol of CO₂ · mg (dry weight)⁻¹ · min⁻¹ was recorded at 3°C and represents an average over 5.2 h under conditions that did not allow renewal of sulfide. Inocula for two other in situ experiments (dives 1967 and 1968) contained considerable amounts of sediment. As a result, the incorporation rates, calculated per dry weight, were extremely low and are not recorded here.

**Enzymological studies.** In one suspension containing narrow Beggiatoa sp. and flexibacteria (suspension 1615B, Table 1), RuBisCO activity was maximal at approximately 45°C (Fig. 4). A significant decline in activity was observed only above 55°C. At 30°C, the phosphoribulokinase activity of this vent Beggiatoa sp. was 6.7 nmol of CO₂ · mg of protein⁻¹ · min⁻¹.

By comparison, a pure culture of Beggiatoa strain 81-6 showed an activity profile which was displaced toward cooler temperatures by approximately 10°C (Fig. 4). Sustained growth of this strain did not occur above 38°C. The maximum specific activity of RuBisCO for this chemoautotrophically grown culture was approximately three times the highest value measured in the vent Beggiatoa spp.

Owing to the limited quantity of frozen material, other experimental suspensions were assayed only at 30 and 50°C. For suspensions containing only narrow Beggiatoa sp. (dives 1612, 1615, and 1970), the RuBisCO activity at 50°C was 1.6 to 4.8 times higher than at 30°C (Table 2). By contrast, the mesophilic marine strain (81-6) showed only 27% of its 30°C activity at 50°C. Suspensions 1606B and 1968, dominated by medium and wide Beggiatoa filaments, respectively, possessed activity at 30°C but no detectable activity at 50°C; however, the 30°C activity was close to the limit of detection for suspension 1606B.

**Microscopy and autoradiography.** Light microscopy of narrow Beggiatoa filaments (Fig. 5A) and of a wide filament (Fig. 5B) showed extensive inclusions of sulfur. Electron microscopy of a sectioned narrow filament (Fig. 5C) revealed the hollow structure of these cells. The relatively small amount of cytoplasm is distributed as a layer attached to the cell wall. There is no clear indication of a membrane separating the cytoplasm from the inner liquid vacuole of the cell, and no cytoplasm extends toward the cell walls. The layered gram-negative cell envelope is shown in the transmission electron micrograph of Fig. 5D which was taken from a wide filament. The balls of cytoplasm suggest extensive membrane intrusions as described by Maier and Gal lardo (21) in their studies of Thiothrix species. The unharmed state of the cell envelope as well as the active metabolism and gliding of the freshly collected filaments argue against a drastic morphological change during sample retrieval or fixation.

Autoradiographs demonstrate extensive ¹⁴CO₂ fixation by Beggiatoa filaments of both width classes contained in suspension 1966 (Fig. 6A and B). No grain development could be observed in the flexibacteria-like filaments (Fig. 6A, lower left), an indication that they lack the capacity for CO₂ fixation. In 48°C incubations, no CO₂ fixation could be demonstrated by autoradiography.

**Cell volumes and protein values.** The cell volumes of all width classes are recorded in Table 1 for the various filament

![Fig. 4. Activity of RuBisCO (nanomoles of CO₂ per milligram of protein per minute) versus assay temperature. Symbols: ■, vent Beggiatoa spp. equivalent to suspension 1615B; ○, Beggiatoa strain 81-6, a mesophilic chemoautotroph. Note different activity scales.](http://aem.asm.org/)

<table>
<thead>
<tr>
<th>Suspension</th>
<th>RuBisCO activity at 30°C</th>
<th>RuBisCO activity at 50°C/RuBisCO activity at 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1606B</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>1612A</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>1612B</td>
<td>1.9</td>
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<tr>
<td>1612C</td>
<td>1.4</td>
<td>2.1</td>
</tr>
<tr>
<td>1615A</td>
<td>1.3</td>
<td>4.8</td>
</tr>
<tr>
<td>1615B</td>
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<td>1.9</td>
</tr>
<tr>
<td>1968</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Strain 81-6 (control)</td>
<td>6.3</td>
<td>0.27</td>
</tr>
</tbody>
</table>

*a* Nanomoles of CO₂ per milligram of protein per minute.

*b* No activity detected at 50°C.
suspending matrix. The hollow nature of the majority of cells in these suspensions is demonstrated as follows. The total protein per milliliter of suspension was measured and compared with an estimated protein value which was derived from (i) the Beggiatoa spp. biovolume per milliliter of suspension and (ii) the assumption that this volume had the protein content of average bacterial cells. The measured protein values of the vent Beggiatoa suspensions were only 9 to 32% of the corresponding estimates (Table 3). In contrast, in 12 separate suspensions of Beggiatoa strain 81-6 (exponential to stationary growth phases) the ratio of measured protein/estimated protein was 110 ± 14%.

When unicellular bacteria enumerated by epifluorescence microscopy were assumed to be cylinders 1 μm in diameter by 2 μm in length, they never constituted more than 1% of the total filamentous biovolume. There was no evidence that any of our cleaning procedures decreased the proportional abundance of unicellular bacteria within the suspension of Beggiatoa filaments.

**TABLE 3.** Comparison between estimated protein and measured protein in various filament suspensions

<table>
<thead>
<tr>
<th>Suspension</th>
<th>Total biovolume (cm³/ml)</th>
<th>Estimated protein (mg/ml)²</th>
<th>Measured protein (mg/ml)²</th>
<th>Measured protein/estimated protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1606A</td>
<td>1.8 × 10⁻²</td>
<td>2.0</td>
<td>0.34</td>
<td>17</td>
</tr>
<tr>
<td>1612A</td>
<td>3.6 × 10⁻³</td>
<td>0.38</td>
<td>0.12</td>
<td>32</td>
</tr>
<tr>
<td>1612C</td>
<td>7.7 × 10⁻³</td>
<td>0.85</td>
<td>0.25</td>
<td>29</td>
</tr>
<tr>
<td>1615A</td>
<td>3.4 × 10⁻³</td>
<td>0.37</td>
<td>0.11</td>
<td>30</td>
</tr>
<tr>
<td>1615B</td>
<td>1.7 × 10⁻¹</td>
<td>19</td>
<td>3.2</td>
<td>17</td>
</tr>
<tr>
<td>1968</td>
<td>1.9 × 10⁻¹</td>
<td>20</td>
<td>1.8</td>
<td>9</td>
</tr>
<tr>
<td>1970</td>
<td>3.1 × 10⁻²</td>
<td>3.4</td>
<td>0.50</td>
<td>15</td>
</tr>
</tbody>
</table>

² Sum of cell volumes from Table 1.

² Based on biovolume of previous column assuming that wet cell density = 1.1 g/cm³, dry weight = 20% of wet weight, protein = 50% of dry weight.

² Directly measured by method of Bradford (2) for identical suspension.

**DISCUSSION**

**Width classes of Beggiatoa spp.** Filament width was previously the sole criterion by which species of the genus Beggiatoa were designated (20). Filaments of the largest width class previously described (B. gigantia) measured 25 to 55 μm across. Currently, only a single species (width, 2 to 4 μm) is recognized (35) because larger representatives have not been characterized in pure culture studies.

In a study on natural samples of Beggiatoa spp., Jørgensen (15) observed that the widest filaments, ranging from 25 to 30 μm, were distinctly set apart from the continuum of narrower filaments. This size corresponds closely to the narrowest type we observed. Klav (18) observed that Beggiatoa widths divided into two subpopulations: 14 to 21 μm (φ = 18 μm) and 26 to 49 μm (φ = 34 μm).
The latter range spans two of the width classes observed in our study. While it is reasonable to consider a 40-μm-wide *Beggiatoa* filament a different species than one of 2-μm width, there is no agreement with respect to width discontinuities observed in natural samples. According to the available literature, our data demonstrate the most clearly separated size classes. The largest ranges from 90 to 160 μm (x̄ = 116 to 122 μm) and has not been reported from other locations.

Lithoautotrophic potential. The narrow *Beggiatoa* filaments dominating suspensions from dives 1612, 1615, and 1970 appear to be sulfide-oxidizing lithoautotrophs. Since they compose at least 95% of the biovolume in these suspensions (Table 3), their RuBisCO activities (Fig. 4; Table 2) are of the same magnitude as that of known lithoautotrophs. The rates of CO₂ incorporation were sometimes strongly stimulated by sulfide (Fig. 2B and 3). Prince et al. (31) suggest that these bacteria possess novel enzymes for sulfide oxidation. They indicate that most of the soluble c-type cytochromes from a bulk collection of filaments identical to suspension 1615B represent a high-molecular-weight complex (M₀ 210,000) which appears to have appropriate redox properties to be involved in the sulfide metabolism. Redox titrations suggested four thermodynamically distinct hemes (Eₘ₇ + 140, +15, −160, and −340 mV).

The wide filaments dominant in suspension 1968 also appear to be autotrophic. Their total cell volume is 100 times larger than that of the 27-μm-wide filaments (Table 1), and the RuBisCO activity (at 30°C) of this suspension is roughly comparable to that of the suspensions dominated by the 27- to 32-μm-wide *Beggiatoa* sp.

Less can be concluded about the autotrophic potential of the 40-μm-wide filaments in the suspensions of dive 1606 (Table 1). The very low RuBisCO activity in the single sample tested (1606B, Table 2) might have been due to prolonged cleaning of filaments resulting in cell lysis. However, other cleaned suspensions (e.g., 1612B and -C) showed higher activities. Since the filaments of dive 1606 were obtained from the surface of sediments rich in both sulfide and organic compounds (mainly low-molecular-weight fatty acids [33; C. Martens, unpublished data]), they might well have been growing litho- or organoheterotrophically or as facultative autotrophs with their RuBisCO activity severely repressed by organic compounds.

Even after making allowance for temperature differences, whole-cell rates of CO₂ incorporation (Fig. 2 and 3) are lower than RuBisCO activities of the corresponding suspensions (Table 2; Fig. 4). Failure to duplicate in situ conditions in the shipboard incorporation experiments is the most likely explanation for this since natural populations and pure cultures of *Beggiatoa* spp. are known to require low and very specific concentrations of oxygen and soluble sulfide for growth (16, 26).

Unusual properties of vent *Beggiatoa* spp. Certain enzymes are known to have a higher temperature optimum and a higher temperature maximum in cell-free systems than is reflected by growth of whole cells (4, 29). We found this to be true in our control strain 81-6, which has an upper growth limit of 38°C and an upper limit of the RuBisCO activity at approximately 52°C (Fig. 4). Some of our data suggest that the narrow *Beggiatoa* filaments of dives 1612, 1615, and 1970 are similar to each other and moderately thermophilic in their temperature optimum (Table 2; Fig. 4). For these samples, the optimum range and maximum temperature of RuBisCO activity are approximately 10°C higher than those of the mesophilic marine control strain. The comparative data of Fig. 4 are very similar to those presented by others (7) for RuBisCO activity of a moderately thermophilic *Chromatium* sp. (30°C optimum) and a mesophilic control strain.

The whole-cell CO₂ incorporation data reported (Fig. 3) seem to be at odds with the enzymological data indicating thermophily because no significant or sustained incorporation was detected above 40°C. Thus, the elevated optimum and upper-temperature limits observed for RuBisCO activity of the vent *Beggiatoa* sp. might be interpreted as an adaptation to the wide temperature fluctuations of the hydrothermal habitat. On the other hand, the absence of the above-mentioned specific microenvironments might make determination of temperature optima difficult in shipboard experiments.

The virtual monoculture of the 28- to 32-μm-wide *Beggiatoa* filaments occurred in unusually heavy layers (up to 60
cm thick) on the outside of hot smoker chimneys within dense populations of vestimentiferan tube worms (Riftia sp.). Compared with the population thickness of 0.5 mm obtained in gradient cultures of Beggiatoa sp. or near-shore sediments (16, 25), the massive growth observed in this hydrothermal habitat is astounding and holds a number of clues for further studies. Three major factors must contribute to the mass occurrence of Beggiatoa spp.: the particular rate and constancy of H$_2$S supply by diffusion or percolation through the chimney wall; the particular temperature during mixing of the reduced and warm hydrothermal fluid with the oxygenated and cold (3°C) ambient seawater; and the possible presence of dissolved organic materials secreted by the vestimentiferan tube worms.

**Morphology.** Electron micrographs (Fig. 5C and D) indicated the existence of large vacuoles lacking cytoplasm which probably contain liquid. Using light microscopy, this feature was previously detected by others (3, 19) in bacteria indistinguishable from Beggiatoa spp. The consistently low ratio of measured protein/estimated protein for suspensions dominated by all three width classes (Tables 1 and 3) strongly suggests that this is a property general to these large Beggiatoa filaments—a point not readily established by microscopy alone. Maier and Gallardo (21) observed similarly large vacuoles in two newly described marine species of Thioploca which, like the Beggiatoa filaments from the Guaymas Basin, have not been isolated in pure culture. Other morphological similarities between Beggiatoa spp. and Thioploca spp. are pointed apical cells and the multilayered cell wall. The only major difference appears to be the common sheath which surrounds several filaments in Thioploca spp. Gallardo (6) also commented on the co-occurrence of a flexibacterium within the dense Thioploca mats observed on the sea floor.

The vacuoles in Thioploca spp. are “surrounded by a thin cytoplasmic shell containing sulfur inclusions,” a characteristic not readily apparent in the Beggiatoa spp. The existence of large central vacuoles may allow these organisms to develop into unusually wide filaments without incurring diffusional limitation of nutrients to the cytoplasm. Thus, these extremely large, rigid, and gliding organisms may be able to exploit a much more extensive environmental space and to form much thicker mats than the narrow Beggiatoa filaments observed in near-shore sediments. The latter can extend at most a few millimeters above the sediment/water interface (22).

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**LITERATURE CITED**


