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# High Nitrate Concentrations in Vacuolate, Autotrophic Marine *Beggiatoa* spp.†

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**Massive accumulations of very large *Beggiatoa* spp. are found at a Monterey Canyon cold seep and at Guaymas Basin hydrothermal vents. Both environments are characterized by high sediment concentrations of soluble sulfide and low levels of dissolved oxygen in surrounding waters. These filamentous, sulfur-oxidizing bacteria accumulate nitrate intracellularly at concentrations of 130 to 160 mM, 3,000- to 4,000-fold higher than ambient levels. Average filament widths range from 24 to 122 μm, and individual cells of all widths possess a central vacuole. These findings plus recent parallel discoveries for *Thioploca* spp. (H. Fossing, V. A. Gallardo, B. B. Jorgensen, M. Huttel, L. P. Nielsen, H. Schulz, D. E. Canfield, S. Forster, R. N. Glud, J. K. Gundersen, J. Kuver, N. B. Ramsing, A. Teske, B. Thamdrup, and O. Ulloa, Nature (London) 374:713–715, 1995) suggest that nitrate accumulation may be a universal property of vacuolate, filamentous sulfur bacteria. Ribulose bisphosphate carboxylase-oxygenase and 2-oxoglutarate dehydrogenase activities in the *Beggiatoa* sp. from Monterey Canyon suggest in situ autotrophic growth of these bacteria. Nitrate reductase activity is much higher in the Monterey *Beggiatoa* sp. than in narrow, laboratory-grown strains of *Beggiatoa* spp., and the activity is found primarily in the membrane fraction, suggesting that the vacuolate *Beggiatoa* sp. can reduce nitrate coupled to electron flow through an electron transport system. Nitrate-concentrating and respiration potentials of these chemolithoautotrophs suggest that the *Beggiatoa* spp. described here are an important link between the sulfur, nitrogen, and carbon cycles at the Monterey Canyon seeps and the Guaymas Basin hydrothermal vents where they are found.**

Some sulfide-rich marine sediments support massive mats of very large sulfur bacteria. The most spectacular assemblages of sulfur-oxidizing bacteria, belonging to the genera *Thioploca* and *Beggiatoa*, occur (i) below extensive upwelling regions, e.g., off the coasts of Peru and Chile, (ii) at deep-sea hydrothermal vents, and (iii) at certain sulfide-rich cold seeps.

The genera *Thioploca* and *Beggiatoa* delineate filamentous bacteria that deposit globules of $S^0$ intracellularly (in the periplasm) at up to 30% of total dry biomass (21, 22). All are capable of gliding at a few micrometers per second on a solid or semisolid surface. Some of the wider *Beggiatoa* spp. are indistinguishable from *Thioploca* spp. except that, in the latter, numerous separate filaments are contained within a single common sheath (19). Although groups of wide filaments ranging from 20 to 160 μm have been observed, no pure culture of *Thioploca* or of *Beggiatoa* strains wider than 4 μm exists.

In a recent study of *Thioploca* spp. from the coast of Chile, Fossing et al. (8) reported that these widely distributed bacteria which achieve great biomass density are able to concentrate nitrate to 500 mM in a liquid vacuole occupying >80% of the cell volume. The filaments were postulated to glide within the sheath, transporting nitrate 5 to 10 cm down into the sediment, where the sulfate reduction rate is 25 mmol $m^{-2}$ $day^{-1}$. There, they were presumed to reduce the nitrate with concomitant oxidation of hydrogen sulfide. This coupling of sulfur and nitrogen cycles by *Thioploca* spp. may be equal to a significant fraction of global pelagic denitrification (8).

The massive populations of *Beggiatoa* spp. observed at hydrothermal vents and certain cold water seeps reach similar densities. At the Guaymas Basin vents, *Beggiatoa* spp. of three discrete width classes occur in mats up to 30 cm thick which fill spaces between clumps of tubeworms (*Riftia pachyptila*) and in mats 2 to 3 cm thick on sediments percolated with warm, sulfide-rich hydrothermal fluids (10, 14, 26). At a sulfide-rich (2) cold water seep in the Monterey Canyon, sediment cores revealed a *Beggiatoa* sp. in similarly dense mats. The sulfide emanating from these seeps is believed to be produced largely by sulfate-reducing bacteria fueled by organic matter present in the seep water.

The current study investigated the nitrate content and autotrophic potential of wide, vacuolate cells of a *Beggiatoa* sp. collected from a Monterey Canyon seep and from the Guaymas Basin hydrothermal vents. Comparison of the present results with results published for Chilean *Thioploca* spp. indicates that all populations of vacuolate, filamentous sulfur bacteria examined to date share the ability to concentrate nitrate from ambient levels by at least 3,000-fold. This property is without precedent in the microbial world and is of potential ecological significance.

## MATERIALS AND METHODS

**Sample collection, maintenance, and preparation.** Monterey *Beggiatoa* samples were collected from a depth of 900 m at the Clam Field Seep (2) off the coast of Monterey, Calif., with the remotely operated vehicle *Ventana*. Sediment cores with at least 10 cm of overlying seawater were collected in August 1994, November 1994, and March 1995 and transported on ice to Davis, Calif., where they were stored at 4°C for up to 2 weeks, the duration of the experiments.

Tufts of a *Beggiatoa* sp. were harvested from cores with Pasteur pipettes and gently washed twice with filter-sterilized, $N_2$-sparged natural seawater (8,000 × *g*, 1 to 2 min, for nitrate concentration assays; 500 × *g* for enzyme assay preparation). After washing, the loose pellets were condensed with a 5-s spin at

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TABLE 1. Site characteristics for benthic locations of mats dominated by large filamentous sulfur bacteria of various widths[a]

| Site[b] | Location | Depth (m) | Temp (°C) | Dominant bacteria | Filament widths[c] (μm) | References |
|---|---|---|---|---|---|---|
| Clam Field Seep | Monterey Canyon, Calif. | 900 | 4 | *Beggiatoa* sp. | 65–85 | This study |
| Guaymas Basin HTV | Gulf of California | 2,000 | 3–~20 | *Beggiatoa* spp. | 24–32, 40–42, 116–122 | 10, 26 |
| OMZ sediments (15–40°S) | Continental Shelf, Peru and Chile | 50–300 | ~10 | *Thioploca* spp. | 12–20, 30–43 | 8, 15a, 20 |

[a] Individual cell lengths ranged from 5 to 36 μm, and they formed filaments 1 to 10 cm long.
[b] Abbreviations: HTV, hydrothermal vents; OMZ, oxygen-minimum zone.
[c] For Guaymas Basin, ranges listed represent average values of discrete width classes for 10 separate samples. Other ranges represent the entire width spectrum observed for a single sample.

16,000 × *g*, the supernatant was decanted, and the pellets were processed as described below.

Vent *Beggiatoa* spp. were collected in 1988 from Guaymas Basin vent site (27°01′N, 111°24′W; 2,004-m depth) with the submersible *ALVIN* (dive 1968). Collection, concentration, and storage procedures are described elsewhere (26). Long-term storage was at −80°C. Nitrate measurements were performed on vent *Beggiatoa* spp. in May 1995. A portion of a frozen pellet was chipped off, suspended in deionized water, and assayed for nitrate concentration as described below.

*Beggiatoa* strains 75-2a and 81-1c were grown in liquid media as described previously (11). Cells were harvested by centrifugation and resuspended in deionized water or enzyme assay buffer as appropriate. Cells were assayed fresh (strain 75-2a) or following storage at −80°C (strain 81-1c).

**Measurement of nitrate and oxygen concentrations.** Cell pellets used for nitrate measurements were resuspended in deionized water and stored at −20°C for less than 1 week. Prior to assaying, samples were subjected to several cycles of freezing (solid $CO_2$ and ethanol) and thawing. Microscopic inspection confirmed breakage of all cells. Aliquots were removed for protein determination, and the remaining cell extract was clarified by centrifuging at 12,000 × *g* for 2 min. All samples and dilutions were filtered (0.2-μm pore size) before injection onto the ion chromatograph (Dionex Corporation; Omnipac Pax-500 analytical column and anion micro-membrane suppressor). The nitrate concentration was calculated on the basis of the peak areas compared with the standard curve generated with $NaNO_3$ solutions in deionized water. Utilizing biovolume/protein ratios (see below), intracellular nitrate concentrations were calculated.

Seawater samples were collected from the surface to 2,000 m in the region of Monterey Canyon with Niskin bottles attached to a conductivity, temperature, depth (CTD) rosette and analyzed for nitrate by colorimetric techniques modified from the method of Strickland and Parsons (28). The partial pressure of oxygen was measured 0.25 m above the sea floor with a Seabird polarographic oxygen electrode attached to the remotely operated vehicle.

**Protein and biovolume determination.** Biovolumes of Monterey Canyon *Beggiatoa* filaments and of unicellular contaminants were determined as described previously (24) on samples washed in filter-sterilized, $N_2$-sparged natural seawater and preserved with 1.5% glutaraldehyde. Parallel frozen samples were assayed for total protein concentration.

The protein in each sample was precipitated with 10% trichloroacetic acid, dissolved in dilute NaOH, and assayed (26) by the Coomassie brilliant blue dye-binding technique of Bradford (3).

**Enzyme assays. (i) Nitrate reductase.** The nitrate reductase assay procedure was based on the protocol described by Lowe and Evans (17), with methyl viologen used in place of benzyl viologen as the electron donor and the potassium phosphate buffer concentration at 150 mM.

Washed Monterey *Beggiatoa* cell pellets were resuspended in dialysis buffer (150 mM $KH_2PO_4$ [pH 7.0], 1.1 mM $Na_2S_2O_4$, 2.4 mM $NaHCO_3$) supplemented with 1.6 mM methyl viologen. The cell suspension was lysed by several freeze-thaw cycles and brief pulses of sonication at 25°C.

The lysate was centrifuged for 5 min at 24,000 × *g*. To remove nitrate and nitrite released by cell breakage, the cell extract (approximately 2 ml) was dialyzed (10,000-molecular-weight cutoff) against 250 volumes of chilled buffer, which was stirred continually and sparged with $N_2$ gas throughout the dialysis. Dialysis buffer was changed after 1 h, and the sample was dialyzed in fresh buffer for an additional 30 min.

The dialyzed cell extract was centrifuged for 1 h at 5°C and 135,000 × *g*. The resulting supernatant (soluble fraction) was stored on ice until assayed. The pellet (particulate fraction) was resuspended in dialysis buffer supplemented with methyl viologen and stored on ice.

Enzymatic activity was taken as the amount of nitrite formed over a 10-min period at 25°C. Nitrite was then quantified by adding the diazo coupling reagents and measuring the colored product spectrophotometrically. *Escherichia coli* nitrate reductase (product N-0519; Sigma Chemical Co., St. Louis, Mo.) was used as a positive control. Throughout the lysis, dialysis, and assay procedures, sufficient $Na_2S_2O_4$-$NaHCO_3$ solution (23 and 48 mM, respectively) was added to maintain a reduced environment as judged by the blue color of reduced methyl viologen. All protein calculations were corrected for these periodic dilutions.

*Beggiatoa* strains 75-2a and 81-1c were processed and assayed as described above, with the following exceptions. Strain 75-2a cells were resuspended in 150 mM $KH_2PO_4$ buffer alone and broken in a chilled French pressure cell ($1.1 \times 10^5$ kPa), and cell extracts of strains 75-2a and 81-1c were not dialyzed prior to ultracentrifugation.

**(ii) RuBPC/O.** Washed filaments resuspended in assay buffer (100 mM Tris hydrochloride, 20 mM $MgCl_2$, 5 mM $NaHCO_3$, 6.5 mM dithiothreitol [pH 8.2]) were passed twice through a chilled French pressure cell ($1.1 \times 10^5$ kPa) and centrifuged (12,000 × *g*, 4°C) to remove debris. The resulting extract was assayed for ribulose bisphosphate carboxylase-oxygenase (RuBPC/O) activity at 5, 15, or 25°C as described elsewhere (23).

**(iii) 2-Oxoglutarate dehydrogenase.** Cells suspended in assay buffer (50 mM $K_2HPO_4$ [pH 8.0]) were broken with freeze-thaw cycles and sonication and centrifuged (12,000 × *g*) to remove debris. Enzyme activity was assayed colorimetrically as described by Brown and Perham (4).

## RESULTS

Mats collected from the surface of cores obtained from the Clam Field Seep (Monterey Canyon) were approximately 1 to 2 cm thick and contained *Beggiatoa* sp. filaments with very little attached sediment material. The *Beggiatoa* filaments were 65 to 85 μm wide (average, 75 μm; $n = 30$) (Table 1) and contained intracellular $S^0$ globules. Mat samples consisted almost entirely of a *Beggiatoa* sp.; all other bacterial cells enumerated by epifluorescence microscopy ranged from 0.2 to 0.9% of the total *Beggiatoa* biovolume. Mat material from the Guaymas Basin hydrothermal vent site (dive 1968) was dominated by a single width class of *Beggiatoa* sp. (range, 88 to 140 μm; average, 118 μm; $n = 39$). Here, a narrower vacuolate *Beggiatoa* sp. (average, 27 μm) and unicellular contaminants each contributed less than 1% of the total bacterial biovolume (26).

Intracellular nitrate concentrations of the large *Beggiatoa* spp. from Monterey Canyon seeps and Guaymas Basin hydrothermal vents were 160 and 130 mM, respectively (Table 2). These concentrations are averages including both cytoplasmic and vacuolar volumes. Relative to ambient nitrate concentrations of 40 μM in the overlying seawater at both sites, this represents a 3,000- to 4,000-fold concentration increase. The intracellular nitrate concentration of the control strain 81-1c was only 0.3 μM, much lower than ambient nitrate levels.

Nitrate reductase in samples of the Monterey *Beggiatoa* sp. was extremely oxygen sensitive, yielding very low activities when not assayed in the presence of reducing agent (data not shown). In the complete assay, the specific activity of particulate nitrate reductase for this vacuolate bacterium was at least 20-fold greater than the corresponding activity for marine and freshwater controls (Table 3). For the vacuolate *Beggiatoa* sp., the ratio of soluble to particulate nitrate reductase was roughly 1:2. In contrast, the calculated ratio for the nonvacuolate controls was at least 4:1.

RuBPC/O activity in Monterey *Beggiatoa* samples ranged from 7.5 to 15.0 nmol of $CO_2$ fixed $min^{-1}$ mg of $protein^{-1}$ ($n = 2$) when assayed at 25°C (Table 4). Consecutive assays at 5, 15, and 25°C showed that specific activity increased monotonically with temperature, yielding values of 6.5, 7.9, and 15.0 nmol of $CO_2$ fixed $min^{-1}$ mg of $protein^{-1}$, respectively. Activity of 2-oxoglutarate dehydrogenase was below the limit of

TABLE 2. Evidence that uncultured, wide *Beggiatoa* and *Thioploca* filaments are hollow, occupy microoxic environments, and accumulate nitrate[a]

| Site and bacterium[b] | Filament width (μm) | Central vacuole by EM[c] | Protein/biovolume (mg of protein/cm$^3$) | Ambient $[O_2]$ (μM) | Nitrate concn | | References |
|---|---|---|---|---|---|---|---|
| | | | | | Ambient (μM) | Intracellular | |
| Narrow control *Beggiatoa* sp. | | | | | | | |
| Strain 81-6 | 4 | No | 121 ± 17 ($n$ = 12) | ~5 | | | |
| Strain 81-1c | 2 | No | | ~5 | 1,000 | ≤0.3 μM | 22a, 25, 26, this study |
| Monterey Canyon *Beggiatoa* sp. | 65–85 ($\bar{x}$ = 75) | ND[d] | 24 | 8–11 | 40–45 | 160 ± 20 mM ($n$ = 5) | This study |
| Guaymas Basin HTV *Beggiatoa* sp. (dive 1968) | 88–140 ($\bar{x}$ = 118) | Yes | 9.5 | ~28 | 0–40 | 130 ± 10 mM ($n$ = 3) | 5, 10, 13, 26, this study |
| OMZ, Peru and Chile | | | | | | | |
| *Thioploca araucae* | 12–20 | Yes | ND[d] | 0–<5[e] | ~25[e] | 150–500 mM[e] | 6, 8, 20 |
| *Thioploca chileae* | 30–43 | Yes | ND[d] | | | | |

[a] Narrow, nonvacuolate, pure culture marine *Beggiatoa* strains 81-1c and 81-6 are included as controls.
[b] Abbreviations: HTV, hydrothermal vents; OMZ, oxygen-minimum zone.
[c] EM, electron microscopy.
[d] ND, not determined.
[e] These data were reported collectively for both *Thioploca* species.

detection (approximately 5 nmol of product min$^{-1}$ mg of protein$^{-1}$) for the Monterey *Beggiatoa* sp. under the conditions tested (Table 4).

The water column nitrate concentration at 600 to 900 m was 40 to 45 μM, and the oxygen concentration in this oxygen-minimum zone (9) was 8 to 11 μM at 0.25 m above the sea floor.

## DISCUSSION

The *Beggiatoa* sp. from cold seeps in Monterey Canyon appears to be very similar to the *Beggiatoa* sp. from hydrothermal vents in the Guaymas Basin and the *Thioploca* spp. found off the coast of Peru and Chile. All are unusually wide filaments, and all are able to concentrate nitrate intracellularly by 3,000- to 20,000-fold, relative to ambient nitrate concentration. There are at least three discrete width classes of filaments (averaging 24 to 32, 40 to 42, and 115 to 122 μm) at the Guaymas Basin vents (Table 1). Electron microscopy revealed that all of these plus Chilean *Thioploca* spp. contain a large central vacuole constituting approximately 80% of their cross-sectional area (14, 20, 22a, 26). On the basis of protein/biovolume ratios, *Beggiatoa* filaments from the Guaymas Basin contained approximately 8% of the protein of nonvacuolate strains (Table 2), agreeing well with the electron microscopy data. Although the Monterey *Beggiatoa* sp. has not been examined by electron microscopy, we assume it contains an equally large central vacuole on the basis of a comparably low protein/biovolume ratio (Table 2), falling in the middle of the range (9.5 to 33 mg of protein per cm$^3$) observed for 10 samples of vacuolate, vent *Beggiatoa* spp. (26).

The significant RuBPC/O activities measured for *Beggiatoa* spp. from the Guaymas Basin (26) and Monterey Canyon and high sulfide concentrations of both environments strongly suggest a lithoautotrophic mode of growth for these organisms. The high temperature optimum for RuBPC/O (25°C) in *Beggiatoa* samples from Monterey Canyon suggests that the organism is not psychrophilic, despite an ambient temperature of 4°C. The lack of 2-oxoglutarate dehydrogenase activity in the Monterey *Beggiatoa* sp. implies that cells are not oxidizing organic matter to an appreciable extent in their natural environment. This enzyme is never detected in an obligate chemoautotroph (strain 81-1c) (Table 4) but is highly regulated in a narrow, facultatively heterotrophic *Beggiatoa* strain (strain 81-6) (Table 4), only showing detectable activity when exogenous organic matter is being oxidized (11). Determination of whether the Monterey *Beggiatoa* sp. is obligate or facultative in its use of an inorganic energy source requires additional studies.

The nitrate reductase activity of the Monterey *Beggiatoa* sp. is higher than that of other *Beggiatoa* strains tested. Unlike other strains, nitrate reductase of the Monterey *Beggiatoa* sp. was very oxygen sensitive. The majority of the activity was found in the particulate fraction of the Monterey *Beggiatoa* sp. cell extract. Respiratory nitrate reductases are typically membrane bound (32), while assimilatory nitrate reductase activity is generally soluble (31). This suggests that the Monterey *Beggiatoa* sp. is able to use nitrate as a terminal electron acceptor in respiration, in contrast to the narrow, nonvacuolate strains. The enzyme subunit compositions of respiratory nitrate reductase enzymes are similar regardless of whether the bacterium denitrifies or produces ammonia as a final waste product (2a). The ease with which nitrate reductase activity (alpha and beta subunits) can be released from membrane vesicles of the denitrifier *Paracoccus denitrificans* (1) is consistent with the signif-

TABLE 3. Nitrate reductase activity in cultured and native *Beggiatoa* spp.

| Strain | Enzyme form | Enzyme activity (nmol/min/mg of protein) |
|---|---|---|
| Monterey *Beggiatoa* sp. | Soluble | 200 ± 76 |
| | Particulate | 395 ± 42 |
| 81-1c | Soluble | 10 ± 1 |
| | Particulate | 0 |
| 75-2a | Soluble | 65 ± 2 |
| | Particulate | 17 ± 3 |

TABLE 4. Activity of RuBPC/O and 2-oxoglutarate dehydrogenase (OGDH) in marine *Beggiatoa* spp.[a]

| Strain | Metabolic versatility | Growth conditions | Enzyme activity (nmol of product/min/mg of protein) | | |
|---|---|---|---|---|---|
| | | | RuBPC/O | OGDH | Reference |
| Monterey Canyon *Beggiatoa* sp. | Unknown | In situ | 7.5–15 | 0 | This study |
| Guaymas Basin vent *Beggiatoa* spp. | Unknown | In situ | 5–6 | ND[b] | 26 |
| 81-1c | Obligate autotroph | Sulfide + acetate | 14.1 ± 1.6 | 0 | 11 |
| | Obligate autotroph | Sulfide only | 20.4 ± 2.5 | 0 | 11 |
| 81-6 | Facultative autotroph | Sulfide + acetate | 3.3 ± 0.2 | 153 ± 11 | 11 |
| | Facultative autotroph | Sulfide only | 23 ± 9.2 | 0 | 11 |

[a] Enzyme assays were performed at 25 to 30°C.
[b] ND, not done.

icant activity of the soluble fraction in the Monterey *Beggiatoa* sp.

Among free-living, sulfur-oxidizing bacteria that are obligate chemoautotrophs, only two unicellular forms are proven denitrifiers (27, 30), and neither has been shown to dominate a pelagic or sediment niche. It was suggested previously that natural *Beggiatoa* populations can denitrify. A mat containing a narrow (4-μm wide) freshwater *Beggiatoa* sp. as an unspecified proportion of total bacteria consumed nitrate at approximately 4% of its oxygen consumption rate (29). On the basis of a 48-h conversion of $^{15}N\text{-}NO_3^-$ to $^{15}N\text{-}N_2$ in this possibly highly contaminated bacterial population, it was concluded that the *Beggiatoa* sp. present could denitrify. This same study also reported denitrification on the basis of tenuous data for the very *Beggiatoa* strain (B18LD) reported previously to be incapable of this specific use of nitrate (31).

Concentration of any solute *x*-fold against an environmental gradient costs a cell a minimum of $\log_{10}(X)$ 5.7 kJ/mol (12). Thus, the 4,000-fold-higher intracellular concentration we have observed for the Monterey Canyon *Beggiatoa* sp. relative to the ambient level costs the cells a minimum of 20.5 kJ of free energy per mol of nitrate taken up and can be theoretically viewed as being driven by proton symport. Oxidation of 1 mol of hydrogen sulfide via nitrate respiration (to dinitrogen) yields approximately 750 kJ of free energy (16). Assuming that chemoautotrophic sulfur bacteria are only one-third efficient at capturing that energy in a proton gradient (12) and accounting for the requirement that 1.6 mol of nitrate be consumed per 1.0 mol of sulfide completely oxidized, only about 0.13 mol of nitrate, therefore, must serve as the oxidant to drive the intracellular accumulation of 1.0 mol of nitrate. Thus, the proposed accumulation of nitrate by putatively chemoautotrophic *Beggiatoa* spp. or *Thioploca* spp. faced with low environmental nitrate concentrations (Table 2) is thermodynamically reasonable.

Fossing et al. (8) proposed a unique transport system in the Chilean *Thioploca* spp. It appears that cells accumulate nitrate from the overlying seawater in their vacuoles and migrate down, within their sheaths, to the sulfide-rich sediments where nitrate is postulated to be reduced with concomitant oxidation of hydrogen sulfide. Therefore, *Thioploca* spp. are not dependent on coexistence and simultaneous diffusion of these two substrates. Similarly, wide, vacuolate *Beggiatoa* spp. may not simply rely on diffusion of available compounds; they might concentrate nitrate from seawater in their vacuoles and then move by gliding motility (roughly 200 μm/min [26]) to a sulfide source. On the basis of a RuBPC/O activity of 6.5 nmol of $CO_2$ fixed $min^{-1}$ mg of $protein^{-1}$ at in situ temperature, it can be estimated that the internal nitrate store (160 mM) of the Monterey *Beggiatoa* sp. could support chemoautotrophic growth for approximately 4.8 h under completely anoxic conditions before the nitrate store was depleted. If *Beggiatoa* sp. motility is directed by chemotaxis (15), this would allow a net migration of up to 6 cm.

Certain sulfate-reducing bacteria also appear to be capable of concentrating their electron acceptor (sulfate) several-thousand-fold (7). However, their lower intracellular concentrations (25 mM or less) and lack of a storage vacuole preclude their sustained metabolic dependence solely on this internal store.

Previously, it was proposed that the unprecedented thickness of *Beggiatoa* mats at hydrothermal vents could exist only as a result of thermally driven circulation of $H_2S$ and $O_2$ in seawater through the mat (10). Simple molecular diffusion of oxygen was completely inadequate to support the observed sulfide-oxidizing ability of those *Beggiatoa* mats which, like the Monterey Canyon mats, experience low ambient $O_2$. In light of our findings, it is likely that nitrate accumulation, anaerobic respiration, and gliding motility enable the mats of *Beggiatoa* spp. at vents and in Monterey Canyon to grow to such thicknesses. In addition to ambient bottom water (Table 2) as a source of nitrate for vacuolar accumulation, the steep sediment ammonia gradients at the Guaymas Basin (18) and Monterey Canyon (20a) must be considered. Nitrifying bacteria might be expected to be most active in the microoxic environment where ammonia diffusing from below first contacts oxygen, and the microaerophilic nature of *Beggiatoa* spp. (21, 25) might position them ideally to exploit this cryptic source of nitrate.

The current study extends the work of Fossing et al. (8) to establish that in all instances examined to date (Table 2), wide, vacuolate, filamentous sulfur bacteria accumulate nitrate massively and occur in environments in which the oxygen concentration is low or nil. Together, these data imply a prominent role for nitrate respiration or denitrification in the metabolism of these and perhaps all other such vacuolate bacteria. Furthermore, this study provides enzyme data strongly supporting both this proposed use of nitrate and the chemoautotrophic nature of the bacteria. Although assays were performed on mixed populations, all other contaminants constituted less than 1% of the bacterial biovolume; thus, the very significant nitrate concentrations, RuBPC/O activities, and nitrate reductase activities can be assigned only to the *Beggiatoa* filaments.

Vacuolate, filamentous sulfur bacteria almost certainly represent an important coupling of the nitrogen and sulfur cycles in the sediments they inhabit. In the case of *Thioploca* spp., the coupling appears to drive denitrification at a globally significant rate (8). Although more limited in distribution, the Monterey Canyon *Beggiatoa* sp. provides a more accessible model of the uncultured bacteria catalyzing these transformations.

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