

Distinct pigmentation and trophic modes in *Beggiatoa* from hydrocarbon seeps in the Gulf of Mexico

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ABSTRACT: Bacterial mats, which are comprised of spatially distinct, pigmented and non-pigmented filamentous *Beggiatoa*, are abundant at hydrocarbon seeps on the continental slope of the northern Gulf of Mexico. Samples of both filament types were collected, using the submarine 'Johnson Sea Link', from seeps at water depths of ~550 m. The water-soluble pigment of colored strains was internal to the cells and had an absorbance peak of approximately 390 nm. Sulfur granules in both pigmented and non-pigmented cells indicated that these *Beggiatoa* had the capability of oxidizing H₂S. Non-pigmented filaments were capable of significant CO₂ fixation based on incorporation of CO₂ by whole, live cells and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) assays using cell-free extracts. RuBisCO activities for extracts from non-pigmented cells ranged from 9.92 to 135.35 nmol CO₂ fixed mg protein⁻¹ min⁻¹. Activities varied significantly with temperature and pH. This ability to use CO₂ as the primary carbon source, along with the ability to oxidize H₂S for energy, suggests that these non-pigmented filaments were chemoautotrophic. Pigmented filaments, in contrast, had little CO₂ incorporation ability. RuBisCO activities from pigmented mats ranged from 0.17 to 0.92 nmol CO₂ fixed mg protein⁻¹ min⁻¹. These results suggest that geochemical processes at hydrocarbon seeps create an environment capable of supporting separate chemoautotrophic and heterotrophic (presumably organo-heterotrophic) *Beggiatoa* populations.

KEY WORDS: Bacterial mat · Autotrophy · Heterotrophy · Chemosynthetic community · Digital camera array

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INTRODUCTION

Abundant bacterial mats, which consist of gliding, filamentous, sulfide-oxidizing *Beggiatoa*, are prevalent at hydrocarbon seep sites in the Gulf of Mexico (Larkin et al. 1994). A distinctive characteristic of *Beggiatoa* filaments at hydrocarbon seeps is their large size. Wirsen et al. (1992) found 2 size-classes consisting of 30 to 50 µm and 85 to 108 µm wide filaments, while Sassen et al. (1993) reported filaments as wide as 200 µm. These widths greatly exceed those of *B. gigantea*, which had been the largest known species having filament widths greater than 25 µm. This size range is comparable to that at the Guaymas Basin hydrothermal vents, where *Beggiatoa* as wide as 122 µm have been collected (Nelson et al. 1989a). *Beggiatoa* mats in Gulf

of Mexico seeps include non-pigmented (white) and pigmented (yellow to orange) colonies, which are spatially distinct as a general rule (MacDonald et al. 1989, Sassen et al. 1994, Larkin & Henk 1996, Sen Gupta et al. 1997). The mats occur on sedimentary settings near faults and fractures, covering shallow gas-hydrate deposits, and peripheral to clusters of chemoautotrophic host organisms such as vestimentiferan tube worms *Lamellibrachia* sp. and seep mussels *Bathymodiolus childressi* (MacDonald et al. 1989, 1994, Fisher 1990, Gustafson et al. 1998). The orange pigmentation of some of the mats is not typical of the cytochrome-derived pink color that is commonly seen (Strohl & Schmidt 1984), but its exact origin is still unclear. Mats of pigmented *Beggiatoa* have also been reported from cold seeps in Monterey Canyon (Barry et al. 1996) and

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from sediments adjacent to hydrothermal vents on Endeavour Ridge (Hedrick et al. 1992). The *Beggiatoa* at Gulf of Mexico seeps is unusual because one often observes distinct zonation of pigmented and non-pigmented mats over scales of a few cm.

Sediment beneath *Beggiatoa* mats at hydrocarbon seeps contains high concentrations of hydrocarbon gases, C₁ to C₅, and oil that has undergone at least partial bacterial oxidation (Kennicutt & Sassen 1995, Sassen et al. 1998). Sassen et al. (1994) reported substantially higher concentrations of hydrocarbons in sediments covered with pigmented mats than in those beneath adjacent non-pigmented mats. However, the gradients they observed were hundreds of parts per million over lateral and vertical scales of a few cm. So, it is not clear whether the different pigmentation styles are associated with distinct geochemical environments or with transient pockets and veins of oil and gas in an extremely high-flux setting. The bacterial oxidation of this abundant organic carbon has an important impact on the seep environment. Bacterial oxidation of hydrocarbons causes depletion in the supply of O₂ in the sediment and leads to the bacterial reduction of sulfate and production of H₂S (Aharon & Fu 2000). The sediment at hydrocarbon seeps therefore contains an ample supply of both CO₂ and H₂S in addition to organic compounds in the form of the hydrocarbons and bacterial metabolites derived from heterotrophic consumption of hydrocarbons. Such conditions would support microbial heterotrophy as well as autotrophy. Nelson et al. (1986) demonstrated chemoautotrophic growth by *Beggiatoa* in oxygen-sulfide micro-gradients. The characterization of hydrothermal vent *Beggiatoa* by Nelson, et al. (1989b) included measurements of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity and CO₂ fixation. RuBisCO activities in filaments in the 24 to 32 μm and 116 to 122 μm width-classes were found to be at levels near those of known chemoautotrophs. Carbon isotope analysis reveals seep *Beggiatoa* biomass to have a light δ¹³C value of -27.9‰ PDB (Sassen et al. 1993). This is compatible with typical values seen for chemoautotrophic carbon and lends credence to the idea that seep *Beggiatoa* use CO₂ generated by the bacterial oxidation of hydrocarbons as their carbon source, and oxidize H₂S for energy.

Results reported by Wirsén et al. (1992) indicated significant rates of CO₂ fixation for non-pigmented *Beggiatoa* from Gulf of Mexico seeps, whereas pigmented *Beggiatoa* had 'virtually no fixation activity.' This investigation was undertaken to examine evidence for multiple trophic modes in *Beggiatoa* from hydrocarbon seeps in the Gulf of Mexico and to determine whether distinct trophic modes are associated with filament size or pigmentation.

MATERIALS AND METHODS

Setting. Samples were collected from 2 principal hydrocarbon seeps on the northern continental slope of the Gulf of Mexico (Fig. 1). The deeper site, designated GC185 (27° 44' N, 91° 30' W), consists of an elliptical mound, approximately 750 m long and 500 m wide and elevated approximately 40 m above the surrounding seafloor. Its crest is at a water depth of 570 m. The mound is aligned along a low-angle fault that intersects the seafloor (Cook & D'Onfro 1991) and provides a conduit for hydrocarbons that migrate to the seafloor from deeply buried reservoirs (Kennicutt et al. 1987). An abundant benthic fauna, including vestimentiferan tube worms, seep mussels, and associated heterotrophic animals, colonizes the fault axis along the crest of the mound (MacDonald et al. 1989). The second site, designated GC234 (27° 44' N, 91° 33' W), is similarly colonized by seep fauna, but is situated in a half-graben at water depths of 525 to 550 m (Fig. 1). Thermogenic hydrocarbons, including gases (methane to pentane) and high molecular weight hydrocarbons (liquid oil), are prevalent in surface sediments of both sites (Sassen et al. 1994).

Bacterial mats were widespread at both sites. Typically, non-pigmented mats consist of reticulated strands that extend across the surface sediments in irregular arrays that can be 1 m or more in width. Pigmented mats generally appear denser and more compact. It is not uncommon to find distinct zones of pigmented mat surrounded by a fringe of non-pigmented filaments (Fig. 2A). Pigmented mats were often asso-

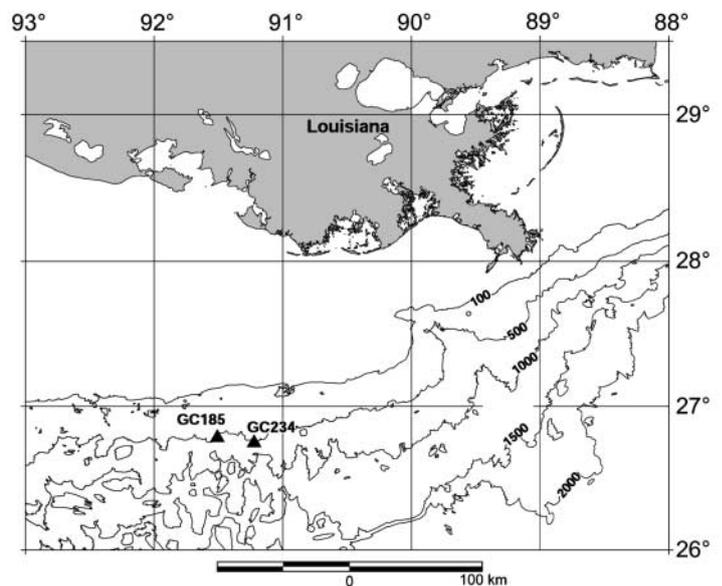


Fig. 1. Study sites in northern Gulf of Mexico (▲); see 'Materials and methods; Setting' for descriptions of sites. Depth contours in m

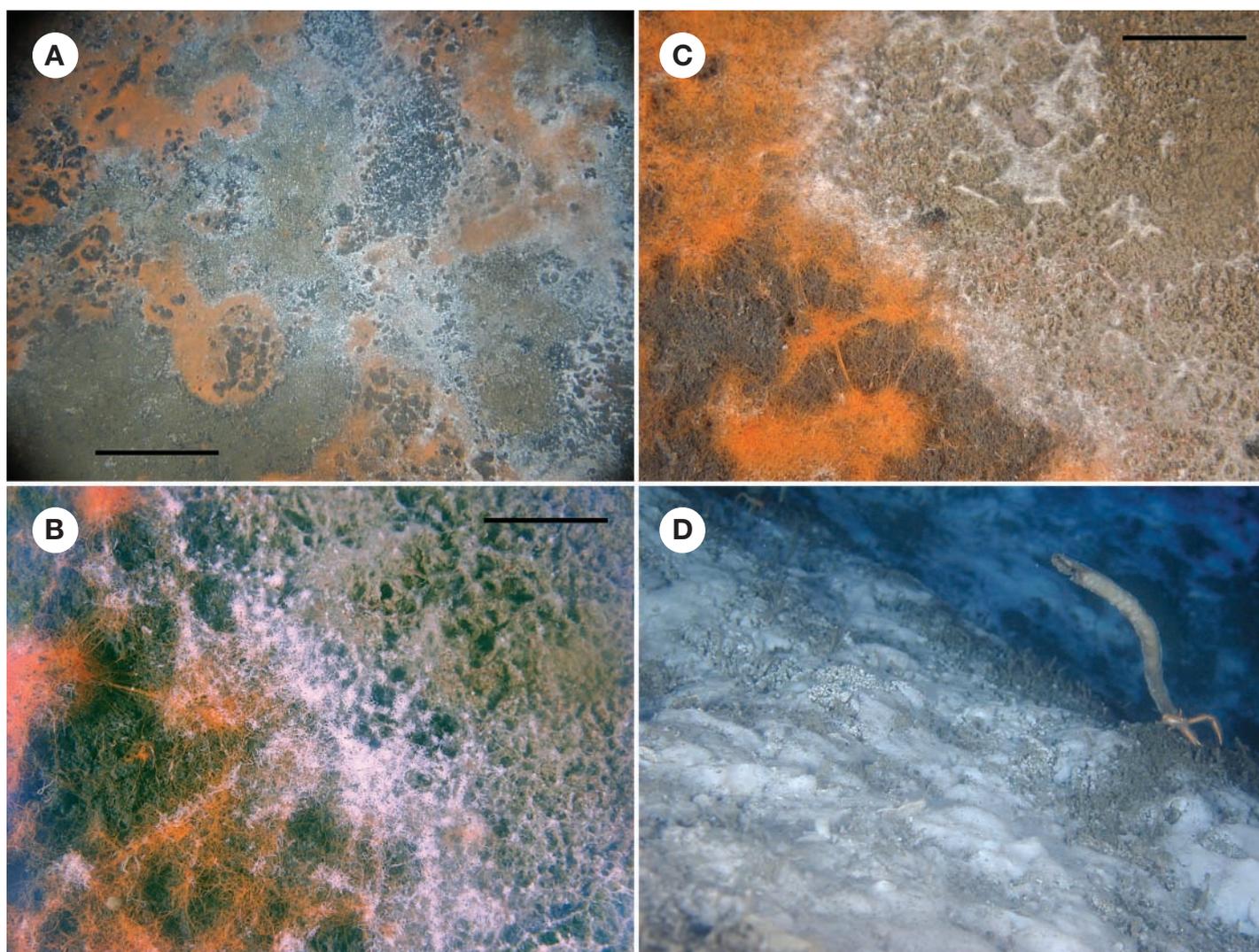


Fig. 2. *Beggiatoa*. Bacterial mats at GC234 study site, northern Gulf of Mexico. (A) Mix of pigmented and non-pigmented mats. Scale bar ~10 cm. (B) Gradient between pigmented and non-pigmented mats. Scale bar ~2 cm. (C) Detail of gradient between pigmented and non-pigmented mats. Scale bar ~1 cm. (D) Detail of amorphous flocculent layer on sediments disturbed by sampling operations. The layer formed in a matter of days following disturbance and covered a total area >15 m²

ciated with shallow or outcropping deposits of gas hydrate (MacDonald et al. 1994, Sassen et al. 1998).

Field methods. Collections of *Beggiatoa* were made using the submarine 'Johnson Sea Link'. High-resolution photographs of mats were taken with a newly designed digital camera array mounted on the submersible arm. The camera, a Nikon Coolpix 990®, was modified to fit in a titanium alloy housing with a flat optical port. A pair of 250 W quartz lamps co-mounted with the housing provided light. Additional illumination for extreme close-ups was supplied by a 36-element LED array built into the lens adaptor. A continuous video signal to the observer in the submersible provided a preview image for frame and focus deter-

mination. Camera function was controlled from the submarine by entering commands on a hand-held computer. The visual distinction between pigmented and non-pigmented mats is generally evident and, where the 2 types co-occur, the gradation from pigmented to non-pigmented filaments occurs over distances of <1 cm (Fig. 2A–C). It was therefore possible to target collections of *Beggiatoa* mats based on their *in-situ* appearance. However, white, flocculent layers of amorphous sulfur, which contained few filaments, were sometimes confused with *Beggiatoa* mats (Fig. 2D). *Beggiatoa* mats were most successfully collected by gently suctioning them off surface sediment, through a hose 10 cm in diameter, and into Plexiglas

containers lined with 50 μm nylon mesh. Non-pigmented mats were collected from Sites GC234 and GC185, and pigmented mats were collected from GC185.

Upon return to the ship, the Plexiglas containers into which mat material was suctioned were immediately placed in the cold room. *Beggiatoa* filaments were then pipetted off the mesh linings and into cryovials. These were then centrifuged in the cold room to obtain moist pellets, which were frozen in liquid nitrogen until later use in protein and enzyme assays. Bacterial filaments were also placed in 50 ml Falcon tubes and preserved in 2% glutaraldehyde for microscopy, including size range and morphology determination. Live filaments were set aside for use in shipboard CO_2 incorporation experiments.

Microbiology methods. Bacterial filaments were identified as *Beggiatoa* based on morphology and size. The *Beggiatoa* width and morphology was determined from preserved and frozen material in which filaments were intact and undamaged. Filaments were wet mounted and viewed using an Olympus BH-2 microscope. One hundred filaments per slide were measured to determine width-frequency in the samples. Bright field photomicrographs were taken using the Olympus PM-10ADS automatic 35 mm camera system attached to the microscope.

Rates of carbon dioxide fixation were determined using fresh, live material. Immediately after collection, live filaments were pipetted into a 15 ml vial containing 14 ml of temperature equilibrated filtered seawater inoculated with 25 μl of 0.25 $\mu\text{Ci ml}^{-1}$ $\text{NaH}^{14}\text{CO}_3$, 10 mM thiosulfate, and 100 μM sulfide. The vial was then incubated in an 8°C water bath for 3 h with 2 ml subsamples taken at 30, 60 and 180 min. The 2 ml subsamples were filtered through 25 mm wide, 0.22 μm Millipore filters using a multiple filtration system. The filters were then placed in scintillation vials and just covered with 0.5 N HCl. The capped and sealed vials were brought back to the lab where any remaining HCl was allowed to evaporate in the fume hood before the addition of scintillation cocktail. Uptake of ^{14}C was then counted with a liquid scintillation counter and converted to nmol CO_2 fixed mg dry weight (DW) $^{-1}$ min $^{-1}$ as described below, substituting mg DW for mg protein. In addition to the samples, a killed control (sample 3 to 4% glutaraldehyde by volume) and live samples lacking thiosulfate and sulfide were also analyzed (Nelson et al. 1989a). The dry weight of mat material contained within the 2 ml subsamples was determined by filtration onto a 47 mm pre-weighed filter, air drying, and weighing. Rates of carbon dioxide fixation were not corrected for the total carbon dioxide of seawater because of the uncertainties in this calculation (see additional information in 'Discussion' section).

RuBisCO activities were measured using cryo-frozen material. Upon return to the lab, bacteria samples stored in liquid nitrogen were transferred to a -80°C freezer. Prior to use, each vial containing a *Beggiatoa* sample was placed in a refrigerator at 4°C to allow the filaments to thaw slowly. Enzyme assays were conducted by the method of Beudeker et al. (1980), with minor modifications for *Beggiatoa*. A subsample was pipetted into a 1.5 ml Eppendorf vial containing assay buffer solution (100 mM Tris buffer, 5 mM dithiothreitol, 20 mM MgCl_2 , 5 mM NaHCO_3 adjusted to a pH of 8.2 at 20°C) in an ice bath. The cells were then sonicated for 5 \times 20 s periods using a sonicator (Branson 25) fitted with a microtip. The resulting cell-free extract was centrifuged for 30 min in a microfuge at 12 500 \times g at 4°C to remove debris. The supernatant (270 μl) was pipetted into a 1.5 ml Eppendorf vial and activated by incubation for 10 min at room temperature. Following activation, 20 μl of a 2:1 mixture of 1 mM 'cold' sodium carbonate and $\text{NaH}^{14}\text{CO}_3$ (57.3 mCi mmol $^{-1}$) solution was added, followed 15 s later by 20 μl of 0.01 M ribulose-1,5-bisphosphate (RuBP), which began the reaction. The vials containing the samples were placed in a 15°C water bath to allow the reaction to proceed at this temperature. Subsamples consisting of 75 μl were taken at 1, 5, 20, and 60 min and placed in separate scintillation vials containing 400 μl of 50% HCl and allowed to sit overnight in the fume hood. After the HCl had driven off any unfixated $^{14}\text{CO}_2$, incorporation of ^{14}C was determined in a liquid scintillation counter. Results were then expressed as nmol CO_2 fixed mg protein $^{-1}$ min $^{-1}$.

In addition to the samples, 3 controls were prepared and assayed as described above. A killed control contained cell free extract to which a final concentration of 5% glutaraldehyde had been added. The killed control was allowed to sit for 10 min prior to addition of $\text{NaH}^{14}\text{CO}_3$. A live control contained active enzyme but lacked RuBP, and the final control contained only assay buffer.

To find the optimum temperature of enzyme activity in the cell-free extract, the above assay was also run at 4, 8, 32, 45, 55, and 60°C. Samples adjusted to a pH of 7.0 at 8, 15, 32, 39, and 45°C were also assayed to account for the change of pH in Tris buffer with changing temperature. The optimum pH was determined by running the assay at pH values of 7.0, 8.2, and 9.0. The bicarbonate and RuBP dependence of RuBisCO activity were investigated by running the assay at NaHCO_3 concentrations ranging from 0.05 to 19.3 mM and RuBP concentrations from 0 to 1.0 M in separate experiments.

The amount of protein in each sample was determined using the Pierce Bicinchoninic acid (BCA) protein assay according to the instructions included with

the assay kit. Since the samples contained the interfering substance DTT (dithio-threitol), an assay procedure using deoxycholate and TCA (trichloroacetic acid) was used to eliminate interfering substances. Following this treatment, the BCA Working Reagent (1.0 ml) was added to the sample which was then vortexed. The samples were then incubated for 30 min at 37°C and allowed to cool to room temperature. The absorbance of each sample was then measured at 562 nm in a spectrophotometer. The concentration of protein in each sample was determined by plotting the absorbance of each sample on a standard curve with protein concentrations ranging from 25 to 400 µg ml⁻¹.

RESULTS

Beggiatoa filament width and morphology

The pigmentation differences were generally very distinct upon close examination of *Beggiatoa* mats (Fig. 2). Preserved non-pigmented *Beggiatoa* material sampled from GC185 contained filaments ranging in diameter from 20 to 95 µm (Fig. 3). The widths observed comprised a continuum of sizes rather than distinct groups. The narrowest filaments (<25 µm wide) and the widest filaments (>85 µm wide) represented a small fraction while more than half the filaments were 25 to 45 µm wide. All of the filaments, regardless of size, were composed of cells that contained an abundance of sulfur granules and exhibited the 'hollow' appearance typical of larger *Beggiatoa* filaments (Larkin & Strohl 1983). Two types of filament were observed: those that were smooth and straight in form and those that appeared knobby and uneven. Complete filaments displayed rounded ends, which are characteristic of this genus. There was no evidence of bundles of filaments contained in a common sheath

or of filaments with tapered ends, as often found in the closely related genera *Thioploca* and *Thiothrix* (Larkin & Strohl 1983, Jorgensen & Gallardo 1999).

Pigmented *Beggiatoa* filaments sampled from GC185 displayed a continuum of widths ranging from 20 to 65 µm (Fig. 3). While the 25 to 45 µm group was again the most abundant, filaments <25 µm wide represented a much greater proportion of the diameters observed than in the non-pigmented sample from the same site. The largest filaments, >55 µm wide, were the least abundant. The pigmented filaments exhibited characteristic rounded ends and seemed to be mostly knobby and uneven in form. It is possible that freezing affected the morphology of some of the filaments that had been frozen. Individual cells appeared hollow and contained numerous sulfur granules. The orange pigmentation of these filaments appeared to be internal and of varying degrees of intensity among the filaments and within individual filaments. This pigmentation, which is unlike the pink cytochrome tint, is soluble in both distilled water and assay buffer, and has an absorbance peak at a wavelength of approximately 390 nm.

Non-pigmented *Beggiatoa* filaments from GC234 were a uniform population 16 to 19 µm wide with a smooth, straight appearance (not shown). These filaments also exhibited characteristic rounded ends and were composed of apparently hollow cells with abundant sulfur inclusions. The filaments appeared to be tinted pink when clustered together, presumably as a result of cytochromes within the cells.

Carbon dioxide incorporation

Shipboard CO₂ incorporation experiments showed non-pigmented *Beggiatoa* filaments from Site GC234 to be capable of appreciable levels of CO₂ uptake (Fig. 4A). The non-pigmented filaments incorporated CO₂ at a rate of 0.017 nmol CO₂ mg DW⁻¹ min⁻¹ at 8°C, the ambient water temperature at the seep sites. This rate increased to 0.033 nmol CO₂ mg DW⁻¹ min⁻¹ when samples were supplemented with 10 mM thiosulfate and 100 µm sulfide. A killed control (3 to 4% glutaraldehyde by volume) also supplemented with 10 mM thiosulfate and 100 µm sulfide showed no CO₂ uptake.

Pigmented *Beggiatoa* filaments from GC234 showed little CO₂ incorporation ability (Fig. 4B). The rate of uptake for unsupplemented filaments was 0.004 nmol CO₂ mg DW⁻¹ min⁻¹ at 8°C. This rate was unchanged at 0.003 nmol CO₂ mg DW⁻¹ min⁻¹ for cells supplemented with 10 mM thiosulfate and 100 µm sulfide. The incorporation rate for a thiosulfate- plus sulfide-supplemented killed control was negligible over a 150 min time-course (0.002 nmol CO₂ mg DW⁻¹ min⁻¹).

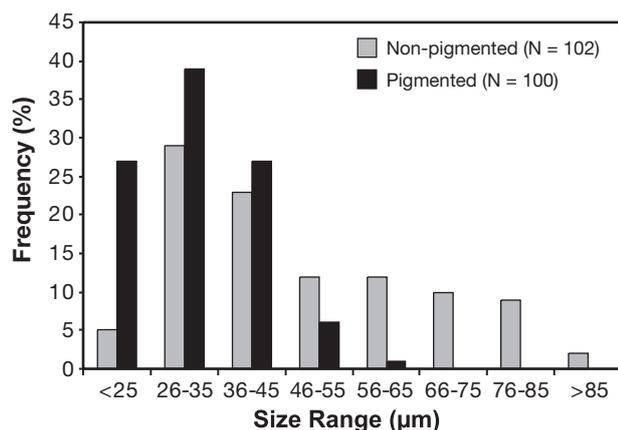


Fig. 3. *Beggiatoa*. Size-frequency distribution of pigmented and non-pigmented filaments from Site GC185

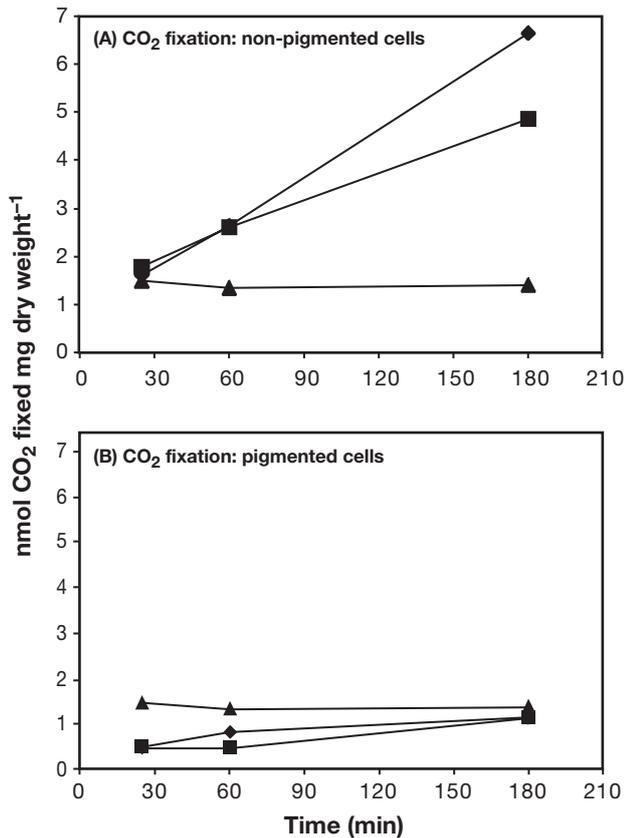


Fig. 4. *Beggiatoa*. Time course determinations of chemosynthetic CO₂ fixation for (A) non-pigmented and (B) pigmented cells. Live and supplemented with 10 mM thiosulfate and 100 μ m sulfide (◆), live unsupplemented (■), killed control supplemented with 10 mM thiosulfate and 100 μ m sulfide (▲)

RuBisCO activity

RuBisCO assays, summarized in Table 1, were initially run at 15°C and a pH of 8.5 with 0.01 M RuBP added to each sample. Under these conditions, cell-free extract derived from non-pigmented *Beggiatoa* filaments from site GC 234 showed significant RuBisCO activity ranging from 7.78 to 13.60 nmol CO₂ mg protein⁻¹ min⁻¹, with an average of 9.65 nmol CO₂ mg protein⁻¹ min⁻¹. Killed controls (5% glutaraldehyde

by volume) for each assay showed no activity. Controls consisting of only buffer solution with no extract added showed virtually no RuBisCO activity (an average of 0.02 nmol CO₂ mg protein⁻¹ min⁻¹). Controls that lacked supplemental RuBP also showed extremely low activity levels (an average of 0.09 nmol CO₂ mg protein⁻¹ min⁻¹). Because all these control values were <2% of the average measured activity, they were not subtracted from the activity.

In contrast, RuBisCO activities measured under identical conditions in cell-free extract derived from pigmented filaments from GC185 yielded RuBisCO activities that were <5% of activities recorded for non-pigmented *Beggiatoa*. Rates for the pigmented extract ranged from 0.18 to 0.57 nmol CO₂ mg protein⁻¹ min⁻¹ with an average of 0.41 nmol CO₂ mg protein⁻¹ min⁻¹ (Table 1). As with the controls from non-pigmented filaments, killed controls and those lacking extract or RuBP showed no RuBisCO activity. However, spinach extract run as a positive control had high RuBisCO activity, ranging from 636.62 to 1618.13 nmol CO₂ mg protein⁻¹ min⁻¹.

Additional RuBisCO activities, measured over a wide range of temperatures, pH values, and RuBP concentrations in both non-pigmented and pigmented filaments, are summarized in Table 1. Mean RuBisCO activities, averaged over all these experiments, were nearly 100 times higher for non-pigmented as compared with pigmented filaments.

RuBisCO activity from non-pigmented filaments was also evaluated over a range of temperatures, pH values, RuBP concentrations, and bicarbonate concentrations. Activity increased with incubation temperature from 4 to 45°C to a maximum activity of 62.19 nmol CO₂ mg protein⁻¹ min⁻¹ (Fig. 5A). RuBisCO activity at 55°C dropped to ca. half of the 45°C rate, and was only 14% of the maximum rate at 60°C. These rates, however, may also have been influenced by the decrease of 0.03 pH units for every 1°C increase in temperature which occurs in Tris buffer. The actual pH at each temperature is shown in Fig. 5A, and the general pattern was similar to the pH-corrected temperature plot shown in Fig. 5B. Samples adjusted to a pH of 7.0 at each temperature showed increasing RuBisCO activity

Table 1. *Beggiatoa*. RuBisCO activities (nmol CO₂ fixed mg protein⁻¹ min⁻¹) for filaments of pigmented (6 assays) and non-pigmented (37 assays) varieties. Uniform assays were conducted at constant pH and temperature

Filament type (assay subset)	N	Temp. (°C)	pH	RuBP (M)	Mean RuBisCO activity	Median RuBisCO activity	Standard deviation
Non-pigmented (uniform)	8	15	8.5	0.01	9.65	9.41	1.97
Pigmented (uniform)	3	15	8.5	0.01	0.41	0.47	0.20
Non-pigmented (all assays)	37	4–60	7–9	0.01–1.0	45.56	40.40	34.45
Pigmented (all assays)	6	8–45	7–8.5	0.01–0.1	0.54	0.52	0.33

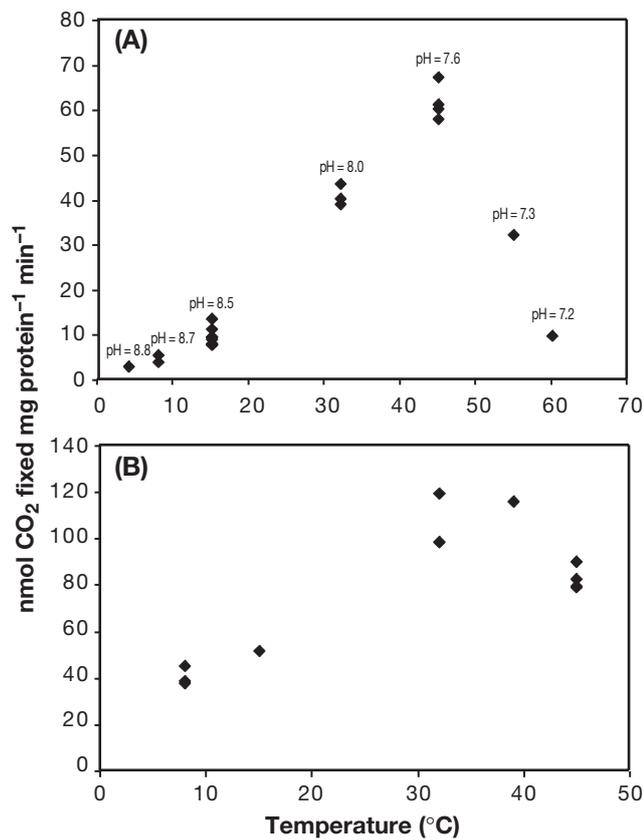


Fig. 5. (A) Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activities versus assay temperature for cell-free extract, where (A) assay buffer varied with temperature (concentration of ribulose-1,5-bisphosphate [RuBP] = 0.01 mol l⁻¹) and (B) pH was adjusted to 7.0 for each temperature (RuBP = 0.10 mol l⁻¹)

from incubation temperatures of 8 to 32°C, where the average activity was 109.03 nmol CO₂ mg protein⁻¹ min⁻¹ (Fig. 5B). At 39°C, activity increased slightly to 115.87 nmol CO₂ mg protein⁻¹ min⁻¹. By 45°C, the RuBisCO activity had dropped to 73% of the 39°C activity level.

In limited experiments, a pH of 7 was optimal for RuBisCO activity in non-pigmented extract, with a maximum activity of 86.43 nmol CO₂ mg protein⁻¹ min⁻¹. Activities at pH values of 8.2 and 9.0, respectively, were only 29 and 13% of the maximal RuBisCO activity. Lower pH values were not examined. Measurement of pH in sediments associated with *Beggiatoa* mats at the 2 sampling sites has detected a range of pH values between 7.4 and 8.1 (MacDonald et al. 2002).

Maximal RuBisCO activity was obtained with RuBP concentrations of 0.1 M or higher, though 0.01 M RuBP yielded more than 80% of maximal activity. RuBisCO activity at NaHCO₃ concentrations from 0.05 to 19.3 mM

increased slowly below 2 mM and more rapidly at higher concentrations. RuBisCO activity did not saturate at the highest bicarbonate concentration used (19.3 mM), so kinetic parameters were not calculated.

DISCUSSION

The range in filament size and morphology for the *Beggiatoa* populations examined in this study was consistent with previous descriptions of *Beggiatoa* from the Gulf of Mexico (Sassen et al. 1993, Larkin et al. 1994). These attributes appear to be characteristic of *Beggiatoa* populations at mid-slope hydrocarbon seeps in this region. The filament sizes were within the range (20 to 150 μm) reported for chemoautotrophic populations of *Beggiatoa* at hydrothermal vents (Nelson et al. 1986), Guaymas Basin hot vents (Nelson et al. 1989a), and cold seeps (McHatton et al. 1996). However, whereas Nelson et al. (1989a) reported 3 distinct width-classes, filament widths of pigmented and non-pigmented filaments in our samples were overlapping and appeared to be normally distributed over the full range. Filament size is an important characteristic in *Beggiatoa* cells because it results from the presence of a large vacuole, which is used to accumulate nitrate from seawater overlying anoxic sediments (McHatton et al. 1996). The vacuole allows *Beggiatoa* to extend its habitat over a greater sediment depth near the sediment-water interface. In our cores, *Beggiatoa* were observed to withdraw 10 cm or deeper into the sediment, probably in response to the collection insult; subsequently the filaments would migrate back to surface sediments. Wirsen et al. (1992) reported high populations of free-living microbes in sediment beneath mats (2.96 × 10⁹ ml⁻¹) and in oily sediments (3.96 × 10⁹ ml⁻¹), indicative of rich organic sediments.

Wirsen et al. (1992) reported CO₂ uptake rates averaging 57.5 nmol CO₂ fixed mg DW⁻¹ h⁻¹ in non-pigmented *Beggiatoa* from seeps in the Gulf of Mexico. The assays were conducted in artificial seawater of known bicarbonate concentration at 8°C, approximately the *in situ* temperature of the *Beggiatoa*. Corresponding assays of orange mat material produced virtually no fixation activity. Without a correction for the total CO₂ in seawater (see below), the rate of CO₂ incorporation in our assays (also at 8°C, Fig. 4) was considerably lower than Wirsen et al. (1992) reported for non-pigmented samples. However, we also found a clear difference in the CO₂ uptake rates between the pigmented and non-pigmented mats, with higher uptake by the non-pigmented mats. In addition, as in Wirsen et al. (1992), the non-pigmented mats responded to supplementation with sulfide and thiosulfate; the pigmented mats did not.

The total dissolved inorganic carbon concentration (DIC) in seawater is about 2 mM, though concentrations in seep areas can be another 0.5 mM higher (Aharon et al. 1992). At seawater pH, however, only a small fraction of the DIC is CO₂ (Aharon et al. 1992). Using the total 2 mM DIC concentration to correct our CO₂ uptake measurements, we calculated high uptake rates for non-pigmented *Beggiatoa* of up to 721 nmol CO₂ fixed mg DW⁻¹ h⁻¹. This rate is more than 10 times that reported by Wirsen et al. (1992) for samples incubated in artificial seawater, though apparently still less than a lab study with freshwater *Beggiatoa* (Grabovich et al. 2001). Regardless of how the CO₂ fixation rate is calculated, the pattern of activity is consistent with the measured RuBisCO activities.

We measured RuBisCO activities in non-pigmented *Beggiatoa* that indicate chemoautotrophic nutrition and are consistent with values reported for chemosynthetic *Beggiatoa* from other environments (Nelson et al. 1989a). The RuBisCO activities for non-pigmented *Beggiatoa* from Gulf of Mexico seeps reported here were much higher than those found by other workers (Wirsen et al. 1992). Positive RuBisCO assays were obtained only when the samples were centrifuged and the pellets frozen under liquid nitrogen. These steps may be crucial for detection of enzyme activities in these populations.

To obtain usable samples in the field, it was also crucial to distinguish *Beggiatoa* mats from white flocculent layers that contained few if any *Beggiatoa*. Dense flocculent layers were developed in a matter of days in areas where the submarine's sampling activities had disturbed the bottom sediment (Fig. 2D). Inspection of fresh collections of floc under a compound microscope revealed no filaments. Laboratory cultures of vibrioid organisms were induced to form similar accumulations of amorphous sulfur by flowing a steady stream of H₂S into organic-rich soils (Taylor & Wirsen 1997). It is probable that disturbance of the bottom enhances the diffusion of H₂S from deeper anoxic layers. The rapid and opportunistic response of a diverse microbial community to transient gradients and venting processes appears to be a hallmark of the organically enriched, but highly heterogeneous seep environment.

These 2 pigmentation types of *Beggiatoa* from the Gulf of Mexico seeps are distinct and reproducible. Pigmented filaments often appeared knobby and less robust than non-pigmented filaments. Although this may have been an artifact of preservation, the effect was different between the filament types and is consistent with observations reported by Wirsen et al. (1992). Abrupt transitions between the 2 varieties strongly suggest competitive exclusion.

Our results support the interpretation that the pigmentation types have trophic significance. There are

3 possible trophic modes for these bacteria. First, the non-pigmented *Beggiatoa* in these settings may be autotrophic organisms that utilize CO₂ as a carbon source and oxidize H₂S for energy. Second, non-pigmented seep *Beggiatoa* may be facultatively mixotrophic in nature, using hydrocarbons or another source of organic material for their carbon supply and H₂S as an energy source. Mixotrophic potential, as defined by Otte et al. (1999), is the use of sulfide or sulfur as an energy source for CO₂ fixation with incorporation of acetate as an additional organic carbon source. Finally, pigmented and non-pigmented *Beggiatoa* may be heterotrophic and not need H₂S for energy, but are able to fulfill both their carbon and energy requirements through oxidation of the seeping hydrocarbons or possibly other organic compounds. The lack of RuBisCO activity and carbon fixation in the pigmented *Beggiatoa* suggests that these cells are probably heterotrophic. Although there has been speculation, based on structural evidence, that pigmented *Beggiatoa* may be capable of methanotrophy (Larkin & Henk 1996), we believe that heterotrophy induced by locally high concentrations of hydrocarbons is a more plausible explanation (MacDonald et al. 2002). However, both pigmented and non-pigmented *Beggiatoa* were found in sediments with high H₂S concentrations and accumulated sulfur granules. This suggests that H₂S remains important for both varieties, even though pigmented *Beggiatoa* may not need it as an energy source.

Facultative mixotrophy might expand the niches available for colonization by non-pigmented *Beggiatoa*. Through their proposed activities of CO₂ fixation, sulfide oxidation, and consumption of hydrocarbon breakdown products, these *Beggiatoa* would also have greater capacity to link and influence the carbon and sulfur cycles at hydrocarbon seep sites than would be the case if they were strictly chemoautotrophic. However, although it is readily possible to collect the 2 pigmentation types for analysis, sampling very easily disturbs micro-gradients in the sediments. Use of techniques such as microelectrodes deployed *in situ* is needed to improve understanding of the geochemical niches that support differently pigmented *Beggiatoa* in Gulf of Mexico hydrocarbon seeps.

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