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Spatial heterogeneity and underlying geochemistry of phylogenetically diverse orange and white *Beggiatoa* mats in Guaymas Basin hydrothermal sediments

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ABSTRACT

Sulfide-oxidizing bacteria of the genus *Beggiatoa* are found in conspicuous, colorful mats on the seafloor above active hydrothermal seeps at Guaymas Basin. Guaymas *Beggiatoa* filaments fall into discrete size classes representing at least five separate 16S rRNA phylotypes, and appear either white, yellow, or orange. During two R/V *Atlantis* cruises to Guaymas Basin, 78 temperature profiles were taken near and within 15 different orange and white *Beggiatoa* mats by the *Alvin* submersible to investigate spatial relationships between mat color and hydrothermal fluid seeps, as indicated by elevated temperatures. The surface temperatures from 78 profiles are similar to each other (on average 8–12 °C, warmer than bare sediments at 3–4 °C), indicating that Guaymas Basin *Beggiatoa* spp., although relying on the hydrothermal system for energy and carbon sources, live within a relatively cool temperature range. Temperatures from 40 cm below orange *Beggiatoa* versus white *Beggiatoa* are the same, at 84 °C averaged across all mat systems. However, within a single mat system, temperatures are higher beneath the predominantly orange center of the mat than beneath the white mat periphery. Push core transects across the orange-to-white color change of three *Beggiatoa* mats showed stronger upward compression of isotherms and metabolic zones beneath the orange mat center than beneath white mat periphery. Hydrothermal temperature gradients push the microbial processes generating carbon and energy sources for *Beggiatoa* mats towards the sediment surface. The resulting steep gradients of hydrothermal electron donors and carbon sources to the sediment surface, rather than the in situ temperature by itself, control the relative positioning of orange and white filaments within a Guaymas Basin *Beggiatoa* mat. Given the wide spectrum of temperature and hydrothermal flux regimes between different mats, the orange/white pattern represents a relative preference or even a competitive balance among different *Beggiatoa* types that establishes itself within each hydrothermal hot spot.

1. Introduction

Seafloor spreading centers are associated with hydrothermal activity and are typically found in open ocean regions with very low sedimentation rates. The Guaymas Basin in the Gulf of California is a unique, relatively near-shore spreading center characterized by hydrothermal fluids that rise through a 300–400 m layer of pelagic and terrigenous organic-rich sediments before escaping at the seafloor (Einsele et al., 1980). Hydrothermal fluids that seep through the thick sediment layer have temperatures greater than 300 °C at depth (Edmond and Von Damm, 1985; Van Damm et al., 1985). The upward transport of methane, organic acids, hydrogen, carbon dioxide, ammonia, and hydrogen sulfide in hydrothermal flow supplies metabolic substrates to a highly diverse microbial community carrying out metabolic processes such as methanogenesis, methane oxidation, nitrification, sulfate reduction, and sulfide oxidation (Dhillon et al., 2003, 2005; Teske et al., 2002, 2009).

Beggiatoa spp. are mat-forming, filamentous, sulfide-oxidizing bacteria that colonize the surface of sulfide-rich sediments. With filament and cell diameters of up to 200 μm, they are among the largest prokaryotes (Schulz and Jørgensen, 2001). At Guaymas Basin, *Beggiatoa* filaments range from only a few μm to more than 100 μm in diameter (Jannasch et al., 1989; Nelson et al., 1989).

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The intracellular volume of Guaymas *Beggiatoa* cells is taken up almost entirely by a large vacuole, while the cytoplasm is only a thin layer between the vacuole and cell membrane (Jannasch et al., 1989; Nelson et al., 1989). In most cases, these large vacuoles accumulate and store nitrate (McHatton et al., 1996), although exceptions are known (Kalanetra et al., 2004). The thin periplasmic space sometimes contains globules of elemental sulfur, and these, along with stored nitrate in the vacuoles, likely sustain filaments during periods of inadequate electron donor or acceptor supply (Strohl et al., 1982; Jannasch et al., 1989; McHatton et al., 1996). In strong contrast to the brown sediments of Guaymas Basin, *Beggiatoa* spp. form conspicuous white, yellow, and orange mats around sites of active hydrothermal venting (Jannasch et al., 1989; Gundersen et al., 1992). The orange pigmentation in *Beggiatoa* filaments is attributed to high cytochrome content (Prince et al., 1988) based on examination of *Beggiatoa* mat sample 1615B, a suspension of brightly colored filaments of a single width class with filament diameters of mostly 25 to 35 μm (Nelson et al., 1989). The predominant cytochrome in at least one orange Guaymas *Beggiatoa* mat has recently been identified as an octaheme cytochrome with homology to hydroxylamine and hydrazine oxidoreductases (MacGregor et al., 2012). The white coloration is due to the refractive nature of elemental sulfur granules stored in the periplasm (Schulz and Jørgensen, 2001). It is currently unclear whether the source of the yellow coloration in *Beggiatoa* mats is intracellular or extracellular. The colorful *Beggiatoa* mats at the surface of otherwise brown sediments are visual markers for complex subsurface microbial communities taking advantage of the electron donor and carbon supply in hydrothermal vents and hydrocarbon seeps (Lloyd et al., 2010). *Beggiatoa* mats with both white and orange filaments show a consistent pattern: orange *Beggiatoa* spp. appear more concentrated than white filaments in the center of the mat, whereas only white filaments occur at the mat periphery. This spatial heterogeneity of *Beggiatoa* filament colors is seen on hot Guaymas Basin sediments and cold Gulf of Mexico sediments (Wirsen et al., 1992; Sassen et al., 1994; Larkin and Henk, 1996; Nikolaus et al., 2003), and suggests different habitat preferences of these *Beggiatoa* types. During two R/V *Atlantis* cruises to Guaymas Basin (December 5–17, 2008; November 22 to December 6, 2009), the association of *Beggiatoa* mats with hydrothermal seepage and the habitat preferences of various *Beggiatoa* types were investigated by geochemical and microbiological characterization of sediments underneath *Beggiatoa* mats in combination with temperature measurements down to 40 cm sediment depth. In particular, temperature profiles and corresponding sulfide, sulfate, dissolved inorganic carbon (DIC), δ^{13}C-DIC, methane, and δ^{13}C-methane gradients from mats exhibiting orange-to-white color transitions were examined to elucidate hydrothermal fluid delivery and/or tolerance associated with differently colored *Beggiatoa* types.

2. Materials and methods

2.1. Isolation of single Beggiatoa filaments, 16S rRNA gene amplification, and phylogenetic identification

On board the R/V *Atlantis* in 1998, *Beggiatoa* filaments collected either by push coring or "slurp gun" from the *Alvin* submersible were selected as single filaments with a pipette and dragged through soft agar (agarose and sterile seawater, 1:1) for about a minute before being stored at −80 °C in 50 μl centrifuge tubes. The orange and white *Beggiatoa* filaments collected in the 1998 cruise were amplified by polymerase chain reaction (PCR) as described previously, without prior whole-genome amplification (Teske et al., 1999). In 2008 and 2009, *Beggiatoa* spp. collected either by push coring or "slurp gun" from the *Alvin* submersible were first diluted into sterile seawater and subsequently diluted into a sterile 0.1% agar in seawater solution. Filaments were then visualized at 40X in a dissection microscope to assess size and color (Table S1, supplemental information). Single filaments were collected by pipetting with a 10 μl pipette tip. Filaments, along with 2–3 μl of sterile fluid, were placed into sterile PCR tubes and frozen at −80 °C. Samples were then transported to the laboratory at UNC-Chapel Hill for further analysis. In Chapel Hill, single filaments were centrifuged briefly and 10 μl of sterile water was added to each tube. After pipetting up and down vigorously to homogenize the filament–water mixture, 5 μl was used for full genome amplification according to the recommended protocol in the Qiagen Ultra-fast miniprep kit (Qiagen, Germantown, MD). From the amplified genome, the gene sequence for the 16S ribosomal RNA was amplified by PCR using the B8F bacterial forward primer (AGR GTT TGA TCC TGG CTC AG) and the B1492R bacterial reverse primer (CGG CTA CCT TGT TAC GAC TT) (Teske et al., 2002). PCR amplifications were run in BioRad iCycler Thermal Cycler (Hercules, CA). Each PCR reaction consisted of 2 μl amplified *Beggiatoa* genome, 2.5 μl 10X FBI buffer (TaKaRa, Shiga, Japan), 2.0 μl dNTP mix, 2.0 μl 10 μM B8F (Invitrogen, Carlsbad, CA), 2.0 μl 10 μM B1492R (Invitrogen), and 0.25 μl SpeedStar Taq polymerase (TaKaRa), and was brought to 25 μl with sterile H_2O. A preliminary melting period of 2 min at 94 °C was followed by 30 cycles of the following steps: 10 s at 98 °C, 15 s at 58 °C, and 20 s at 72 °C. These cycles were followed by 5 min at 72 °C and the final temperature was brought down to 12 °C. Amplifications were confirmed by gel electrophoresis followed by staining with ethidium bromide and visualization under ultraviolet light. Amplicons were cloned using the TOPO TA cloning kit (Invitrogen) and 3–5 individual colonies per filament were sent to GeneWiz (South Plainfield, NJ) for sequencing.

Near-complete 16S rRNA gene sequences were analyzed using Sequencher (Gene Codes, Ann Arbor, MI) and confirmed as closely related (95% maximum identity) to other non-Guaymas *Beggiatoa* spp. via the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/). Next, the sequences were incorporated into a 16S rRNA alignment with sequences from related Gammaproteobacteria using the ARB phylogeny software package (Ludwig et al., 2004) and the SILVA v95 database (Pruesse et al., 2007). A phylogenetic tree was constructed using ARB's neighbor-joining function with Jukes–Cantor correction. Sequences were deposited at NCBI Genbank with accession numbers JN793553 through JN793557 (Table S1, supplemental information).

2.2. Temperature profiling

During *Alvin* dives 4483–4492 (Dec 6–17, 2008) and 4562–4573 (Nov 22–Dec 6, 2009) in the 2000 m deep Southern Guaymas trench, 113 temperature profiles were taken in sediments near and within *Beggiatoa* mats, at the hydrothermally active areas from 27°N00.30 to 27°N00.60, and 111°W24.65 to 111°W24.35 (Table S2, supplemental information). All temperature probe measurements, positions of the probe in the mat, and penetration depths were checked with the *Alvin* dive videotapes that provide a continuous record of all dive operations. Of the 113 temperature profiles, 78 were measured in mats with both orange and white filaments to focus on the relationship between differently colored *Beggiatoa*. A Heatflow probe manufactured by the Woods Hole Oceanographic Institution (WHOI) was used to measure 69 of the 78 temperature profiles. This is a 0.6 m titanium tube containing a linear heater and five thermistors (type 44032, Omega Engineering, Inc.) at 10 cm intervals along the length of the tube (personal communication with Lane J. Abrams, WHOI).