Time-series analysis of two hydrothermal plumes at 9°50′N East Pacific Rise reveals distinct, heterogeneous bacterial populations

J. B. SYLVAN,1 B. C. PYENSON,2 O. ROUXEL,3,4 C. R. GERMAN3 AND K. J. EDWARDS1

1Marine Environmental Biology Section, Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA
2Haverford College, Haverford, PA, USA
3Woods Hole Oceanographic Institution, Woods Hole, MA, USA
4Université Européenne de Bretagne, Université de Brest, Institut Universitaire Européen de la Mer (IUEM), UMR 6538, Institut Français de Recherche pour l’Exploitation de la Mer (IFREMER), Plouzané, France

ABSTRACT

We deployed sediment traps adjacent to two active hydrothermal vents at 9°50′N on the East Pacific Rise (EPR) to assess the variability in bacterial community structure associated with plume particles on the timescale of weeks to months, to determine whether an endemic population of plume microbes exists, and to establish ecological relationships between bacterial populations and vent chemistry. Automated rRNA intergenic spacer analysis (ARISA) indicated that there are separate communities at the two different vents and temporal community variations between each vent. Correlation analysis between chemistry and microbiology indicated that shifts in the coarse particulate (>1 mm) Fe/(Fe+Mn+Al), Cu, V, Ca, Al, 232Th, and Ti as well as fine-grained particulate (<1 mm) Fe/(Fe+Mn+Al), Fe, Ca, and Co are reflected in shifts in microbial populations. 16S rRNA clone libraries from each trap at three time points revealed a high percentage of Epsilonproteobacteria clones and hyperthermophilic Aquificae. There is a shift toward the end of the experiment to more Gammaproteobacteria and Alphaproteobacteria, many of whom likely participate in Fe and S cycling. The particle-attached plume environment is genetically distinct from the surrounding seawater. While work to date in hydrothermal environments has focused on determining the microbial communities on hydrothermal chimneys and the basaltic lavas that form the surrounding seafloor, little comparable data exist on the plume environment that physically and chemically connects them. By employing sediment traps for a time-series approach to sampling, we show that bacterial community composition on plume particles changes on timescales much shorter than previously known.

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Corresponding author: K. J. Edwards. Tel.: 213 821 4390; fax: 213 740 8123; e-mail: kje@usc.edu

INTRODUCTION

Hydrothermal plumes form at deep-sea hydrothermal vents, where hot hydrothermal fluids are injected into the water column and mix with ambient seawater. The hot, buoyant hydrothermal fluids rise in the water column as they mix with cold, oxygenated seawater until a level of neutral buoyancy is attained, typically after a timescale of ~1 h and after dilution of the vent fluid ~10^4:1 by surrounding seawater (Lupton et al., 1985). At this point, neutrally buoyant plumes become disperses by local deep ocean currents and can readily be detected through elevated particle concentrations as well as elevated hydrothermal constituents, such as 3He, Mn, CH4, and NH4+ over distances spanning several kilometers away from the vent source (Lilley et al., 1995; Cowen et al., 1998).

The microbiology of hydrothermal plumes has received less attention than that of the source fluids emanating from chimneys and active hydrothermal chimneys themselves. Given that the entire volume of the oceans cycles through hydrothermal plumes on timescales of just a few thousand years (comparable, for example, to the timescale for mixing of the entire volume of the deep ocean through thermohaline circulation), this environment warrants greater attention (Elderfield & Schultz, 1996; German & Von Damm, 2004). Cell counts in hydrothermal plumes are consistently higher than background waters (Sunamura et al., 2004). Mn oxidation in

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hydrothermal plumes is elevated compared with background seawater, up to 2 nM h⁻¹ (Cowen et al., 1986; Dick et al., 2009). Oxidation of NH₄⁺ and CH₄ is also prevalent in hydrothermal plume environments, whereas these processes are generally absent or occur at very low levels in deep water masses outside of plumes (De Angelis et al., 1993; Lam et al., 2008). These combined evidences for microbial processes in the plume environment indicate that it is a potentially unique microbial biome.

The chemistry of hydrothermal plumes is directly related to the end-member chemistry of the hydrothermal fluids from which the plumes originate. The spatial and temporal variability of vent fluid chemistry at 9°N EPR is among the most thoroughly studied of any vent field. Multiyear time-series of annual to semi-annual sampling at this site have revealed both temporal variability between samplings and differences between vents that are tens of meters apart (Von Damm, 2004). It would be expected that the microbial community in the hydrothermal plumes resulting from these variable end-member fluids may also vary on short temporal and spatial scales, but less is known about the microbial communities at 9°N than the fluid chemistry at any comparable resolution of study. Sampling of hydrothermal plumes is often carried out using shipboard CTD rosettes, but sediment traps have recently been employed to collect samples in time-series of hydrothermal plume fallout at the Endeavour Segment of the Juan de Fuca Ridge (JdFR) (Roth & Dymond, 1989; Cowen et al., 2001), 13°N EPR (German et al., 2002) and 9°N (Adams et al., 2011; Bennett et al., 2011). While those studies examined the impact of hydrothermal plumes on deep-sea geochemistry, they did not study plume microbiology. However, their time-series approach to sampling hydrothermal plumes did reveal that particulate organic carbon is elevated in plumes at distances of a few kilometers from the vent sites (Roth & Dymond, 1989; Cowen et al., 2001) and that plumes are important to the cycling of hydrothermally derived metals (Fe, Cu, Zn) and seawater-derived oxyanions (P, V, As) as well as particle-reactive elements (REE, Th) (German et al., 2002).

Here, we have explored the use of sediment traps for a time-series approach to understanding spatial and temporal effects on the bacterial communities within hydrothermal plumes at 9°N EPR following the eruption in Winter 2005–2006 (Tolstoy et al., 2006). That eruption resulted in elevated dissolved CH₄ in hydrothermal plumes at 9°N EPR when compared with typical hydrothermal plume concentrations, 1 month prior to our deployment (Cowen et al., 2007). Our study, spanning July–November 2006, included a collaboration with both larval biologists, who have already reported evidence for a very disrupted contemporaneous larval supply compared with what was previously present at this site (Mullineaux et al., 2010), and geochemists who have investigated chemical flux variations at both sites using major and trace element analyses, stable and radiogenic isotope measurements, and organic carbon measurements (Bennett et al., 2011). Furthermore, the present study builds on previously published mineralogical and bio-inorganic chemical investigations of similar vent-trap samples to those that are studied here which hypothesized that microbial populations in plumes at 9°N EPR produce the organic ligands that bind hydrothermally produced Fe²⁺, inhibiting its oxidation (Toner et al., 2009).

**MATERIALS AND METHODS**

**Site description and sample collection**

The two sediment traps (McLane Parflux Mark 78H-21; McLane Research Laboratories, Falmouth, MA, USA) with a sampling aperture of 0.5 m² (80 cm diameter at top, 2.5 cm diameter at bottom) and 21 cups were deployed during the RESET06 cruise AT15-6 aboard the R/V Atlantis II, June/July 2006, as described previously (Mullineaux et al., 2010). Briefly, before deployment, cups were filled with a solution of 20% dimethylsulfoxide (DMSO) in ultra-pure water saturated with NaCl and buffered to pH 9.0 ± 0.5. DMSO was used as a preservative for biological samples, and the saturated NaCl solution ensured that particles that fell into the solution, which was denser than seawater, would remain in the sampling cups. The combination of DMSO and saturated NaCl in sediment trap collection cups has been proven successful previously to prevent biological activity while preserving DNA for later study (Comtet et al., 2000). The sampling schedule for both traps was 6 days per bottle, starting on July 01, 2006 (samples R1-1 and R2-1), and ending on November 03, 2006 (Trap R1, sample R1-21), or November 01, 2006 (Trap R2, sample R2-21). Trap moorings were anchored such that the opening of each trap was located 4 m above the seafloor. Following retrieval of Trap R2, it was discovered that a jellyfish had become lodged in the opening at the bottom of the trap during the collection of sample 11 (which began collection on August 30, 2006) and remained there for the duration of the experiment.

Trap R1 was located ~30 m southwest of the Bio9 vent complex within the axial summit caldera in an area with a series of small chimneys, black smokers and spires, which hosts Riftia pachyptila and Tevnia tubeworm populations. Trap R2 was located outside the axial summit caldera, 115 m southwest of Ty and Io vents and ~370 m southwest of Trap R1. Ty and Io are black smokers located ~350 m southeast of Bio9 and are home to populations of Alvinella worms.

Samples were retrieved from the traps, 15 mL was subsampled for microbiology, and all samples were stored at 4 °C until DNA extraction.

**DNA extraction and ARISA**

7.5 mL of each sample was filtered onto a 0.2-µm Nuclepore filter. DNA was extracted using a CTAB phenol/chloroform
procedure (Ausubel et al., 1999). ARISA PCR was carried out according to Hewson et al. (2006). 10 ng DNA was added to each DNA reaction of 2.5 units of RedTaq (Sigma-Aldrich, St. Louis, MO, USA), 10X reaction buffer, 2.5 mM MgCl, 0.2 mM dNTPs, and 0.2 μM of each primer in 50-μL reactions. ARISA primers 78F (5’-GYACACACGGCCCGT-3’) and 79R (5’-[TET]GGGTTBCCCCATT-CRG-3’) were used. The amplification cycle used was: denaturing cycle for 5 min at 95 °C, followed by 30 cycles of 95 °C for 40 s, 56 °C for 40 s and 72 °C for 90 s, and a final extension for 7 min at 72 °C. PCR was treated with Clean and Concentrate Kit-5 (Zymo Research, Irvine, CA, USA). Six nanogram of cleaned DNA per sample was loaded in duplicate into an ABI 377XL Running Slab Gel, and bands were analyzed using ABI GENESCAn software (Applied Biosystems, Foster City, CA, USA). Initial analysis of bands was carried out using Microsoft Excel, and statistical analyses of band ARISA profiles were carried out using PRIMERS (PrimerE, Ivybridge, UK).

Chemical analyses of plume particulates

Trap samples were split into fine size fractions (<1 mm) and coarse fractions (>1 mm) using nylon sieves. Each fraction was later filtered through 47-mm filters, dried under class-100 laminar flow hood, and weighed to determine mass flux. The remaining <1 mm fraction was passed through a 10-port rotating wet sediment splitter. Between 5 and 10 mg dried subsamples were analyzed for Al, Ca, Fe, Mg, Si, Ti, and Mn using an inductively coupled plasma optical emission spectrometer using standard methods established for sediment trap analyses (Honjo et al., 1995).

For each sample, another subsample fraction was dissolved in concentrated acid to determine trace element composition (Ba, Sr, Ga, P, V, Co, Cu, Pb, Zn, 232Th, and 238U) by inductively coupled plasma mass spectrometry (ICP-MS). Subsamples were leached overnight with 10 mL of 8M distilled HF and 3 mL of concentrated HNO3 was then used on a high-resolution Thermo- Electron Element 2 after appropriate dilution (typically 25- to 200-fold dilution depending on the amount of digested materials). The detection limit was calculated from repeat analysis of blank filter digests. Indium was added to each sample as an internal standard to correct for changes in instrument sensitivity. Stock 1000 μg L⁻¹ standards (Specpure, Spex) of each element of interest were diluted in preparation for instrument calibration (ranging from 5 to 1000 ppb). A number of geo-reference standards (BHVO-1 and IFG) were also analyzed along with the samples to confirm analytical accuracy, which is better than 5% for all elements reported.

Statistical analysis of ARISA results with vent chemistry

Spearman’s correlation coefficients between chemical measurements and ARISA profiles were calculated using the BIOENV method in Primer6 with Euclidean distances and 99 permutations. This analysis compares the individual sample chromatograms (input as a grid of samples vs. band size and intensity) from ARISA with the individual chemical variables measured (38 in total) and determines what combination of chemical variables best correlates to the observed ARISA patterns. The BIOENV calculation takes all combinations of the 38 total elemental analyses, from single variables through all possible combinations of each variable, and reports the individual or combination of elemental analyses that yield the highest correlation with the ARISA profiles, regardless of the number of variables that correlate best (therefore, if Al+Ca together have a higher correlation to the ARISA profiles than Al alone and/or Ca alone, it will report the value for Al+Ca). We here report the correlations with the highest values.

Clone library construction and analysis

Universal bacterial primers 27F and 1492R (Lane, 1991) were used to construct near full-length 16S rRNA gene libraries. PCR products were run on an agarose gel, cut out, and extracted using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. Fragments were cloned into the pCR 4 TOPO vector using the TOPO TA Cloning Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. Colonies were randomly selected and grown in liquid culture followed by sequencing at the University of Florida Sequencing Services Facility. Forward and reverse sequences were joined and edited using BIOEDIT v7.0.5 and checked for chimeras using the BELLEROPHON tool of Greengenes (DeSantis et al., 2006b). The resulting sequences were aligned using the Greengenes NAST server (DeSantis et al., 2006a) and imported into ARB (Ludwig et al., 2004). The most similar cultured strains as well as some of the nearest neighbors from ARB for uncultured clones were exported with the sequences from this study and aligned using MEGAS5 (Tamura et al., 2011). Phylogenetic trees were constructed following manual adjustment of this alignment and maximum-likelihood analysis based on the Tamura–Nei model with a Gamma distribution and 500 bootstrap replicates using MEGA5. Calculation of rarefaction curves and diversity estimates, as well as comparison between clone libraries and those of other studies, was carried out using the software MOTHUR (Schloss et al., 2009).
Clones sequenced in this study were deposited in the NCBI database with accession numbers JN873927–JN874385.

RESULTS

Automated rRNA intergenic spacer analysis was used to provide a broad view of major differences in the bacterial community composition from sediment trap samples. Samples R2-11–R2-21 (66–126 days) were considered compromised by the jellyfish caught in the mouth of the sediment trap; therefore, these samples have not been analyzed by ARISA. All other samples from each sediment trap were subjected to DNA extraction and ARISA PCR (n = 31). Successful ARISA PCR amplification was obtained from 13 samples. ARISA results for the bacterial communities from Trap R1 group almost entirely separately from those in Trap R2 (Fig. 1). Sample R2-8 (42–48 days) is the only exception; it is most similar to sample R1-1 (1–6 days). Sample R1-21 (120–126 days) is unique compared with all other samples. Bacterial communities in samples R1-2, R1-8, R1-9, R1-13, R1-17, and R1-19 (6–12, 42–48, 48–54, 72–78, 96–102, and 108–114 days, respectively) all group together, as do samples R2-2, R2-4, and R2-5 (6–12, 18–24, and 24–30 days), indicating that the samples from each trap display a characteristic genetic signature.

We used Spearman’s correlation analysis to determine which environmental variables, measured here as elemental concentrations, best explain the distribution of bacterial communities seen in the ARISA dendrogram (Fig. 1). Both size fractions of each of the 18 elemental concentrations measured in addition to the ratio Fe/(Fe+Mn+Al) were compared with the ARISA analysis using the BIOENV method in Primer6. Multiple combinations of only 11 chemical parameters (the coarse-grained Fe/(Fe+Mn+Al), Cu, V, Ca, Al, $^{232}$Th, and Ti and the fine-grained particulate (<1 mm) Fe/(Fe+Mn+Al), Fe, Ca, and Co) correlate best with differences in the observed distribution of bacterial communities, explaining 71% of the variation (Table 1). Fe/(Fe+Mn+Al) and Ca are the only variables for which a correlation existed for both size fractions.

We cloned and sequenced the near full-length bacterial 16S rRNA gene from traps R1 and R2 at time points 8, 13, and 21 (42–48, 72–78, and 120–126 days, respectively). While samples R2-13 and R2-21 are too compromised for quantitative comparison to the other samples, we use the results generated here to determine bacteria that may be present in the plume at these locations. To avoid artifacts from the experimental disruption, clones from samples R2-13 and R2-21 were included in the analysis of phylum level diversity (Fig. 2) and phylogenetic trees (Figs 3 and 4) only if they met at least one of two following criteria: (i) 97% similarity to at least one clone from samples R1-8, R1-13, R1-21, or R2-8 (not compromised) or (ii) were Epsilonproteobacteria, Zetaproteobacteria, or Aquificales classes of bacteria well represented at hydrothermal vents. These clone libraries allow for comparison between traps and also at each trap over time.

Proteobacteria are the most common clones. Epsilonproteobacteria dominated samples R1-8, R1-13, and R2-8, but are present in all clone libraries. Gammaproteobacteria are the most common clones sequenced in samples R1-21, R2-13, and R2-21. Planctomycetes are present in samples from both traps at time points 8 and 13, but not 21. Other commonly occurring bacterial phyla are Bacteroidetes, Verrucomicrobia, and Cyanobacteria. Clones related to Tenericutes are recovered only from samples R1-8 and R1-13. Clones related to Aquificae are recovered only from samples R1-13 and R2-8.

Because of the high number and diversity of clones sequenced, we demarcated clones by 97% similarity to each other to define representative operational taxonomic units.

### Table 1

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Variables in italics are >1 mm size fraction, variables in bold are <1 mm size fraction. Significance for all correlations is P < 0.05.
OTUs (Schloss et al., 2009). OTUs and even entire classes of bacteria are not evenly distributed between the two sediment traps (Table S1). For example, Firmicutes and Actinobacteria are found in Trap R1, but not in Trap R2. OTUs 113F02 (Planctomycetes), 18F04, 18E03, 18H12 and 18D03 (all Epsilonproteobacteria), and 18B02 (Alphaproteobacteria) are all recovered from samples R1-8 and R1-13, but not from Trap R2. OTU 18B04 (Epsilonproteobacteria) is present in all three samples from Trap R1, but none from Trap R2. OTU 28G07 (Alphaproteobacteria) is recovered only from samples R2-8 and R2-13. These examples all occur in at least two samples; there are many OTUs that are recovered from single samples. It must be noted, however, that the number of clones sequenced per sample was not sufficiently high to draw strong conclusions about the absence of a particular group or OTU, but can be used to discuss general trends in bacterial distributions.

There are also many OTUs recovered from both traps (Table S1). OTU 121A02, a *Marinobacter*, is the most abundant OTU, recovered from samples R1-21 and R2-21. While no OTUs were recovered from all six samples, or even from 5 of 6, many are recovered from 3 or 4 samples including at least one sample from each trap. Nine OTUs are shared by three samples – one Alphaproteobacterium, six Epsilonproteobacterium, one Gammaproteobacterium, and one cyanobacterium (Table S1). Two of these OTUs, both Epsilonproteobacterium (18G01 and 18H01), are shared by four samples (Table S1).

Samples R1-8 and R1-13 share the most OTUs with each other, followed by R1-8 and R2-8 (Table 2 and Fig. S1). R1-8 has the lowest percentage of unique sequences, 23%. Sample R1-21 has the highest (79%); it shares only one OTU with R1-8, two with R1-13, four with R2-21, and none with samples R2-8 and R2-13. Samples R1-8, R1-13, and R2-8 share the most OTUs by absolute number.

By far, the Epsilonproteobacteria represent the most diverse class of bacteria, with 65 OTUs (Fig. 3). Most OTUs group within the orders Sulfurovumaceae or Sulfuricurvaceae, but members of Arcobacteraceae, Hydrogenimonaceae, and Nautillaceae are all present as well. Several Aquificae clones are
Fig. 3 16S rRNA phylogenetic tree of Epsilonproteobacteria and Aquificae clones retrieved in this study. Clones in tree are all <97% similar with all others in this study, as determined by MOTHUR. For a full reporting of all clones, see Table S1. Alignments of sequences from this study and their nearest cultured organisms in the Greengenes database as well as some close uncultured clones from the ARB database were constructed using MEGA5. The tree was constructed using the maximum-likelihood algorithm with 500 bootstraps. Bootstrap values >50% are reported. Aquificae are used as an outgroup. NCBI accession numbers for sequences from cultured representatives and environmental clones are given following the species/clone name.
detected, related to *Aquifex* and *Desulfurobacterium*. Many of the Gammaproteobacterial OTUs recovered (Fig. 4) are most closely related to psychrophiles (*Colwellia*), symbionts (*Photobacterium leiognathi* str. RM1, *Umboniibacter marinipu-

nipes* str. KMM 389, *Calyptogena phaseoliformis* symbiont str. KT-2, and *Codakia orbicularis* gill symbiont), organisms
Table 2: Number of shared OTUs between samples calculated at 0.03% similarity level. Outlined boxes indicate number of OTUs unique to that sample and total number of OTUs for that sample (in parentheses). Note that total number of OTUs is based on rarefaction analysis and therefore biased by sample size.

<table>
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OTU, operational taxonomic unit.

isolated from sediment (Halica sp. str. SY02, Cycloclasticus pugettii str. PS-1 and Kangiella koreensis str. DSM16069) and known sulfur oxidizers (Thioalkalvibrio sp. str. HL-EbGR7). Marinobacter are particularly diverse, forming 18 different OTUs as defined by the 97% cutoff (Fig. S2). A similar pattern was observed for Alphaproteobacterial OTUs falling within the Rhodobacteraceae. OTUs that fell within the Acidobacteria are most closely related to uncultured clones from Loihi Seamount and Kazan Mud Volcano.

Rarefaction curves for the six clone libraries show that the three samples with the highest proportion of Epsilonproteobacteria, R1-8, R1-13, and R2-8, display the steepest rarefaction slopes (Fig. S3). Chao1, ACE, and Inverse Simpson’s diversity indices all indicate R2-8 to be the most diverse sample, followed by R1-13 and R1-8, in agreement with the rarefaction analysis (Table S2). All clones recovered were used for this analysis; therefore, diversity may have been higher in samples R2-13 and R2-21 if there had not been a disruption in sampling.

To test the hypothesis that hydrothermal plume particles comprise a unique deep ocean habitat with endemic bacterial communities, we compared 16S rRNA gene clone libraries from this study with those from basalts (Santelli et al., 2008; Mason et al., 2009; Sudek et al., 2009), deep seawater (Pham et al., 2008; Santelli et al., 2008; Dick & Tebo, 2010), direct hydrothermal fluids (Huber et al., 2006), and hydrothermal plumes (Nakagawa et al., 2005a; Dick & Tebo, 2010). We restricted our selection of studies to those in which universal 16S rRNA primers were used on uncultured samples, and for whom clone frequency was reported. We included all sequences from samples R2-13 and R2-21 for this analysis. We used the Morisita–Horn index of similarity because it accounts for proportion of OTUs in a sample and is not biased by sampling effort, which was different between the studies in this analysis.

Samples from time period 8 and 13 form their own clade (Fig. S5), as do basalts from the EPR, Loihi Seamount, and JdFR. Despite partial blockage of sample R2-13, it still groups with samples R1-8, R1-13, and R2-8. As with the ARISA dendrogram (Fig. 1), analysis of the clone libraries indicates that the communities in the two traps were different from each other – samples R1-8 and R1-13 are most similar to each other as are R2-8 and R2-13. One basalt rock from Vailulu’u Seamount, near Samoa, is more similar to a hydrothermal plume also from the western Pacific. Samples from background water and hydrothermal plumes in the Guaymas Basin look very similar to each other (Dick & Tebo, 2010).

DISCUSSION

Sampling for this study occurred following an eruptive event at the site in January 2006 (Tolstoy et al., 2006), during which lava reached as far as 2 km away from the ridge axis (Soule et al., 2007). Methane concentrations measured in the hydrothermal plume at 9°50’ N EPR during May 2006 revealed concentrations up to 350 nM, 100X greater than typical plume measurements for stable hydrothermal plumes and >4X greater than measurements made after the 1991 eruption (Love et al., 2008). The eruptions killed off much of the resident microbiological communities, and post-eruption larval studies found that resident species had been replaced by vent communities from 300 km away (Mullineaux et al., 2010). Here, we describe bacterial community composition in descending particles from two different active hydrothermal vents at 9°50’ N EPR during a 4-month time-series from August to November 2006. Our samples are the same as those collected by Mullineaux et al. (2010).

Automated rRNA intergenic spacer analysis was used to investigate variability between 13 of the samples collected by the sediment traps. The same methodology has been successfully used to investigate microbial diversity across a deep-sea halocline (Daffonchio et al., 2006), to illustrate a predictable seasonality in surface water bacterial communities collected during a time-series experiment (Fuhrman et al., 2006), and to investigate correlations between bacterial communities and environmental variables in a time-series study (Steele et al., 2011). Here, ARISA shows that bacterial community composition is variable on the timescale of weeks and also the spatial scale between the two vents. Bottles sampled consecutively in the same trap are sometimes less similar than those taken a month or longer apart (Fig. 1). As an example, in Fig. 1, sample R1-2 is much less similar to sample R1-1 than samples R1-9 and R1-13. Similarly, sample R1-8 is much more similar to samples R1-18 than it is to R1-9, which was sampled immediately following R1-8. This is not specific to samples from Trap R1. Sample R2-5 is more similar to sample R2-2 than it is to sample R2-4. The chemistry of hydrothermal vents is known to be variable from year to year, a topic studied extensively at this study site (Von Damm, 2004), and recent work indicates that it is also variable on much shorter timescales (Bennett et al., 2011). While our samples represent particles falling out of a hydrothermal plume, and therefore the observed changes in the microbial community are a result of changes in both the hydrothermal environment (chemistry and microbiology) and also the overlying water column (manifested as particles falling into the traps of a
non-hydrothermal origin, as discussed below), the ARISA results show that bacterial communities on hydrothermal plume particles are also variable over short timescales.

Overall, there is also a difference between the two particle sample sets. Three of four samples from Trap R2 form their own branch on the cladogram (Fig. 1), and seven of nine samples from Trap R1 form a separate branch of the cladogram. This is strong evidence for different communities in the two plumes. There are multiple likely causes for this. First, individual vents appear to harbor unique microbial communities. This is evidenced here and also at Axial Seamount, on the Juan de Fuca Ridge (Opatkiewicz et al., 2009), where individual vents, while variable over a 6-year study period, harbor prokaryotic communities unique to each vent. Also, samples of direct hydrothermal fluid collected from within the vent orifice during the time period of this study revealed that Bio9', which is part of the Bio9 vent complex, had similar Fe concentrations to Ty vent, but only ~40% of the H2S content, yielding higher Fe/H2S (339 at Bio9', 132 at Ty) ratios for the Bio9 vent complex (and resultant plumes) than the Ty/Io area (Bennett et al., 2011). Therefore, we should expect that plumes emanating from different vents potentially contain different bacterial communities.

Additionally, our particle traps were placed at different distances from the vents. Trap R1 was located just 30 m from Bio9 and, hence, would have underlain the most particle-laden lower reaches of the buoyant hydrothermal plume. By contrast, Trap R2 was deployed 115 m from Ty/Io and, hence, may only have underlain the uppermost fringes of the buoyant plume as well as the non-buoyant plume.

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Consequently, it is to be expected that Trap R1 would receive a more significant hydrothermal component of its total input than Trap R2. To examine differences in hydrothermal input between the two traps, we use the ratio of Fe to (Fe + Mn + Al). Fe, Mn, and Al are all elevated in hydrothermal fluids relative to seawater, but Fe/Al and Mn/Al ratios in hydrothermal fluids are well above detrital values (German & Von Damm, 2004). As Mn and Al generally remain in the dissolved phase in the near-vent non-buoyant plume, purely hydrothermal samples contain nearly no particulate Al or Mn but high particulate Fe, so they should exhibit Fe/(Fe + Mn + Al) ratios of ~1. The greater the Mn and Al component, by contrast, the lower the Fe/(Fe + Mn + Al) should be, consistent with a lower hydrothermal input (Boström et al., 1969; German et al., 1990).

For this study, Fe/(Fe + Mn + Al) values reveal that Trap R1 contains almost exclusively hydrothermal input (Fe/(Fe + Mn + Al) ~0.90), whereas Trap R2, as was to be predicted because it was located further from the active vent site, was less influenced by hydrothermal input (Fe/(Fe + Mn + Al) ~0.66; Table S3).

Finally, mineralogical changes can also occur as particles travel down-plume, away from their hydrothermal source. Previous work has shown that sediment traps placed 1.5–20 m and 300 m away from active hydrothermal chimneys at the Totem vent site, 12°N EPR, collected particles with very different compositions – the nearer traps, placed within the summit caldera, collected 4–7 times higher Fe fluxes (derived from hydrothermal input) than the trap placed 300 m away, outside the caldera (German et al., 2002). As hydrothermal particles travel away from their vent of origin, sulfide and sulfate minerals tend to be deposited rapidly while oxy-hydroxide materials disperse further afield; this may potentially influence the bacterial community (Cowen & German, 2003).

In this study, we find a significant correlation between the >1 mm size fraction of Fe/(Fe + Mn + Al), Cu, V, Ca, Al, Ti, and 232Th, the <1 mm size fraction of Fe/(Fe + Mn + Al), Fe, Ca and Co, and bacterial community composition. It is interesting that Fe/(Fe + Mn + Al) and Ca are the only elements for which both size fractions are significant – the former is an indication of the relative proportion of hydrothermal influence in a sample while the latter is likely an indicator of both hydrothermal input (in the form of anhydrite) and, more probably, biogenic (detrital) input from the overlying surface ocean. Al, Ti, and 232Th, by contrast, are all clear indicators of detrital input, presumably from colian deposition that, like any Ca-rich biogenic input, must have settled from the surface ocean. Given the fresh bare-rock settings of these axial vent sites, surrounded by fresh lava-flows (Soule et al., 2007), we do not anticipate that local sediment resuspension is a likely candidate for biogenic or detrital inputs to these traps. Rather, the correlation between microbial communities and indicators of non-hydrothermal (biogenic and detrital) input to Trap R2 most likely reflect the fact that the total hydrothermal input to Trap R2 is lower than to Trap R1 (because it is further from the vent orifice), resulting in a higher degree of dilution of the hydrothermal flux by particles settling from the overlying water column, which can be considered relatively constant, over time, at both sites.

Our results from the cloning and sequencing of 16S rRNA from three concurrent samples in both traps revealed a success in the bacterial community during the course of the experiment. Epsilonproteobacteria were present in all the samples, as is indicative of hydrothermal systems (Sunamura et al., 2004; Nakagawa et al., 2005b; Campbell et al., 2006). Rarefaction curves were steepest for the three samples with the highest proportion of Epsilonproteobacteria (R1-8 was 52%, R1-13 was 60%, and R2-8 was 44% Epsilonproteobacteria). This suggests that the diversity within the Epsilonproteobacteria drives overall diversity within these samples. In other studies, Epsilonproteobacteria are similarly found to be the drivers of intervent diversity at Axial Seamount (Opatkiewicz et al., 2009), and specifically, the presence or absence of Sulfurovum appears to be a major driver for this diversity (Huber et al., 2007).

Clones from the genera Sulfurovum, Sulfurimonas, and Caminibacter dominated our samples (Fig. 3 and Table S1). Sulfurimonas autotrophica and Sulfurimonas denitrificans, the closest cultured representatives to the clones recovered here, are known to chemolithoautotrophically oxidize reduced sulfur compounds under microaerophilic conditions using O2 as an electron acceptor (Inagaki et al., 2003; Sievert et al., 2008). S. denitrificans can additionally use H2 and NO3 as an electron donor and acceptor, respectively. Sulfurovum lithotrophicum also chemolithoautotrophically oxidizes reduced sulfur, but not H2, while reducing O2 or NO3 (Inagaki et al., 2004; Takai et al., 2005). Members of the Caminibacter genus are strictly H2 oxidizers that use O2, S0, or NO3 as electron acceptors (Alain et al., 2002; Miroshnichenko et al., 2004; Campbell et al., 2006) and can grow autotrophically (Caminibacter profundus and Caminibacter mediatlanticus) or mixotrophically (Caminibacter hydrogeniphilus). Members of Sulfurimonas and Sulfurovum can grow at mesophilic temperatures, while members of the Caminibacter genus are moderately thermophilic. Given the lifestyles of these Epsilonproteobacteria, the hydrothermal plume particles collected here, rich in reduced sulfur compounds and containing microniches where microaerophilic conditions can be maintained, are ideal substrates, especially for members of Sulfurimonas and Sulfurovum.

The biggest difference between time points 8 and time points 13 and 21 was an increase in proportion of Gammaproteobacteria and Alphaproteobacteria while the proportion of Epsilonproteobacteria diminished. Many of the Gammaproteobacteria clones from time points 13 and 21 samples were related to known heterotrophic species (Table S4) common to hydrothermal environments. In particular, samples R1-21 and R2-21 are dominated by Marinobacter, a genera known...
to be common in hydrothermal environments (Kay & Baross, 2000; Edwards et al., 2003; Kaye et al., 2011). The presence of these Marinobacter clones in both traps (and therefore not likely due to the jellyfish caught in only Trap R2) indicates a shift in bacterial communities in the plume during this period. The diversity of clones within the Marinobacter genus (Fig. S2) indicates these different OTUs may have different ecological niches. Known niches among cultured representatives of the Marinobacter include Fe-oxidation, hydrocarbon degradation, and a variety of organic degradation pathways (Gauthier et al., 1992; Edwards et al., 2003; Antunes et al., 2007). Similar niche partitioning among species with nearly identical 16S rRNA genes is well documented in marine bacteria (Rocap et al., 2002; Acinas et al., 2004) and may be the case here.

Also present during this last sampling period were clones that fall within Halomonas Group 2A and Group 2C (Fig. S4). Members of Halomonas Group 2A appear to form a clade of subsurface Halomonads, while members of Halomonas Group 2C appear associated with massive sulfide deposits (Kaye et al., 2011), and an iron-oxidizing Halomonas was recently isolated from hydrothermally influenced subsurface crust (Smith et al., 2011). The presence of these organisms is therefore indicative of hydrothermal input, although a shift from the earlier samples.

Some of the Gammaproteobacteria recovered from later samples are indicative of water column populations. These include the clones related to Colwellia pyrtheritraea, P. leognathi, and Pseudoalteromonas sp. str. JL-S1, which are all residents of the deep ocean water column (Table S3). These clones were primarily recovered from Trap R2, which was less hydrothermally influenced than Trap R1 (Table S3).

Most of the Alphaproteobacteria clones recovered fall within the Rhodobacteraceae family, and all of these fall within the Roseobacter clade. A recent survey of 32 Roseobacter genomes revealed that 72% contained the soxB gene, which codes for the SoxB component of the periplasmic thiosulfate-oxidizing Sox enzyme complex and is therefore indicative of the capability for S-oxidation (Newton et al., 2010). Therefore, while not all Roseobacteria are capable of S-oxidation, many are and it is possible that those found here, in an environment with elevated concentrations of reduced S compounds, are among those participating in S-oxidation. In particular, 27 clones related to Sulfitobacter dubius str. KMM 3554 were recovered from sample R2-21. Although only recovered from R2-21, S. dubius is known to oxidize reduced S and therefore is likely a resident of the plume environment.

Few investigations have focused specifically on hydrothermal plume microbiology since the introduction of molecular methods in oceanography. A recent study found that while clones indicative of aerobic methanotrophy were enriched in non-buoyant plume samples from Guaymas Basin vs. background samples, plume and background samples were similar at the class level (Dick & Tebo, 2010). These authors found few Epsilonproteobacteria in the non-buoyant plumes above the hydrothermal field, despite their abundance on the chimneys and sediments on the seafloor in this area. However, they recovered representatives from the SUP05 clade of Gamma-proteobacteria, which are known sulfur oxidizers common in other hydrothermal plumes and oxygen minimum zones (Sunamura et al., 2004; Walsh et al., 2009; German et al., 2010). Perhaps the niche filled by Epsilonproteobacteria at 9°50’N EPR is filled by SUP05 Gammaproteobacteria in the Guaymas plumes.

Another study found Beta- and Gammaproteobacterial ammonia oxidizers in plumes over the Endeavour Segment of the Juan de Fuca Ridge (Lam et al., 2008). It is possible that our lack of detection of bacterial ammonia oxidizers and methanotrophs results from their lack of association with particles. The studies cited above used Niskin bottles to collect their samples at plume depth. Therefore, hydrothermal particles and planktonic cells were both sampled, whereas we collected almost exclusively sinking particles in our sediment traps. Previous work has shown that while there is some overlap between the membership of particle-attached and free-living bacteria in hydrothermal environments, differences do exist, including the elevated presence of Epsilonproteobacteria in the particle-attached population (Huber et al., 2003). Our work confirms the association of Epsilonproteobacteria with hydrothermal particles, even at >100 m away from the venting source of the particles.

It is also possible that the short duration of time the samples could have traveled within the plumes from any vent source to these traps would not be long enough to allow methane- and ammonia-oxidizing microbes to bloom. At the slow lateral current speeds present above the EPR near 9°50’N (∼0.01 m s⁻¹; Thurnherr et al., 2011) and 115 m from Ty and Io vents to Trap R2, it would take at least 3 h to travel the horizontal distance (vertical travel time is not considered because the plume is likely traveling horizontally with the currents as it rises). In that case, because only a few hours might have elapsed from when the fluids left the vent until the particles fell into our sampling devices, the populations reported here may be comprised largely of organisms entrained into the buoyant plume as opposed to those that have developed in situ within the dispersing non-buoyant plume. We might especially expect this to be the case for Trap R1, but to evaluate this further would require additional study involving the deployment of traps farther afield from the EPR 9°50’N vent site and at a greater height off bottom, so that only particles settling from the non-buoyant hydrothermal plume (together with surface ocean biogenic and detrital inputs) could settle into those traps, without any buoyant hydrothermal plume input.

Our analysis of bacterial communities from hydrothermal and deep seawater communities (Fig. S) revealed that samples from EPR plume particles are unique (time points 8...
and 13) from the other communities. They are dissimilar to hydrothermal plume samples collected in Guaymas Basin (Dick & Tebo, 2010), likely due to the differences in host environments (bare-rock vs. sediment-hosted vents) and sampling methods discussed above. Samples RI-21 and R2-21 are most similar to each other and most closely related to background seawater from the deep EPR. The high numbers of Marinobacter and Alphaproteobacteria clones in these samples likely drive this similarity – 19% of the clones in the background deep EPR sample are Marinobacter.

SUMMARY

We found bacterial communities on hydrothermal plume particles at 9°50’N to be heterogeneous on the timescale of weeks. This work illustrates the use of a time-series approach to studying hydrothermal plumes, an environment that is difficult and expensive to sample. By deploying sediment traps to study this environment, we were able to observe bacterial community dynamics on a timescale previously unattainable.

Automated rRNA intergenic spacer analysis revealed that bacterial communities can shift on weekly timescales and that the plume particle bacterial communities were unique at each vent. There is a statistically significant ($P < 0.01$) relationship between elemental concentrations of Fe/(Fe+Mn+Al), Al, Ca, Cu, $^{232}$Th, Ti, V, Fe, and Co and bacterial community structure. 16S rRNA clone libraries mirrored the ARISA distributions in that sample RI-21 was dissimilar from the other samples and showed a progression in bacterial communities during the timescale of this experiment. Earlier clone libraries are dominated by sulfur-oxidizing Epsilonproteobacteria, which diminished in proportional importance in later samples, where more Gammaproteobacteria and Alphaproteobacteria were recovered. The diversity of these samples with Epsilonproteobacteria is higher than those without. These hydrothermal plume particle samples appear to form their own group when compared with other environmental 16S rRNA clone libraries, indicating that the hydrothermal plumes at 9°50’N host a unique community.

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REFERENCES


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Venn diagrams of number of shared OTUs between each pair of samples from this study.

Fig. S2 Expansion of the Marinobacter branch of the Gammaproteobacteria tree (Fig. 4).

Fig. S3 Rarefaction curve for clone libraries from this study, calculated with MOTHUR.
Fig. S4 16S rRNA phylogenetic tree of Gammaproteobacteria clones recovered during this study, with representative Alphaproteobacteria, Zetaproteobacteria and Deferribacteres included to root the tree.

Table S1 Distribution of OTUs in clone libraries from this study. OTUs with an asterisk (*) next to their name are from the group ‘other (non-plume)’ in Fig. 2.

Table S2 Diversity indices as calculated by MOTHUR. Ranges given are 95% confidence intervals for each index.

Table S3 Relative hydrothermal component of the particulates collected (calculated as the ratio of Fe to (Fe+Mn+Al)).

Table S4 Ecophysiology of Zetaproteobacteria, Alphaproteobacteria and Gammaproteobacteria clones recovered from time points 13 and 21.

References

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