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Prokaryotic Populations in Arsenic-Rich Shallow-Sea Hydrothermal Sediments of Ambitle Island, Papua New Guinea

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This study is the first to investigate the microbial ecology of the Tutum Bay (Papua New Guinea) shallow-sea hydrothermal system. The subsurface environment was sampled by SCUBA using push cores, which allowed collection of sediments and pore fluids. Geochemical analysis of sediments and fluids along a transect emanating from a discrete venting environment, about 10 mbsl, revealed a complex fluid flow regime and mixing of hydrothermal fluid with seawater within the sediments, providing a continuously fluctuating redox gradient. Vent fluids are highly elevated in arsenic, up to ∼1 ppm, serving as a "point source" of arsenic to this marine environment. 16S rRNA gene and FISH (fluorescence in situ hybridization) analyses revealed distinct prokaryotic communities in different sediment horizons, numerically dominated by Bacteria. 16S rRNA gene diversity at the genus level is greater among the Bacteria than the Archaea. The majority of taxa were similar to uncultured Crenarchaea, Chloroflexus, and various heterotrophic Bacteria. The archaeal community did not appear to increase significantly in number or diversity with depth in these sediments. Further, the majority of sequences identifying with thermophilic bacteria were found in the shallower section of the sediment core. No 16S rRNA genes of marine Crenarchaeota or Euryarchaeota were identified, and none of the identified Crenarchaeota have been cultured. Both sediment horizons also hosted "Korarchaeota", which represent 2–5% of the 16S rRNA gene clone libraries. Metabolic functions, especially among the Archaea, were difficult to constrain given the distant relationships of most of the community members from cultured representatives. Identification of phenotypes and key ecological processes will depend on future culturing, identification of arsenic cycling genes, and RNA-based analyses.

Keywords arsenic biogeochemistry, iron biogeochemistry, hydrothermal vents, sediment microbiology

INTRODUCTION

Shallow-sea hydrothermal vent systems (HVSs) occur in a variety of tectonically active settings. Commonly associated with submarine volcanism, island and intra-oceanic arcs, ridge environments, areas of intraplate oceanic volcanism, and less commonly at continental margins and rift basins, these systems can be regarded as intermediate environments between deep-sea and terrestrial hydrothermal systems (Tarasov et al. 2005). Shallow-sea HVSs are characterized by distinct redox fronts between oxic seawater and anoxic vent fluid, at the interface of the subsurface and subaerial biospheres. In addition, hydrothermal mixing that occurs in the transition from hydrothermal vents to lacustrine and marine fluids generates a repository of potential sources of energy for chemolithotrophic microbial growth (Amend et al. 2003, 2004; Rusch et al. 2005). These systems are often characterized by complex microbial communities, consisting of aerobes and anaerobes, thermophiles and mesophiles, archaea and bacteria, and they serve as windows to the subsurface biosphere.

Although shallow-sea HVSs have received increasing attention in recent years (see, for example, the special issue of *Chemical Geology*, 2005, vol. 224), most studies focus either on the geology/geochemistry or on the microbiology;

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concurrent collection of detailed geochemistry and microbiology data remains relatively rare. The prokaryotic communities have been catalogued for only a few geochemically described shallow-sea HVSs, most notably off-shore Vulcano and Panarea (Aeolian Islands, Italy), Milos (Aegean Sea, Greece), Taketomi (Japan), and Eyjafjordur (Iceland) (Marteinsson et al. 2001; Maugeri et al. 2009; Rusch and Amend 2008; Sievert et al. 1999, 2000a). The geochemistry of these environments has been at least partially characterized, indentifying temperature and pH ranges, mineralogy, gas composition, or redox disequilibria for potential metabolisms (Amend et al. 1998, 2003; Marteinsson et al. 2001; Rogers and Amend 2005; Sievert et al. 1999, 2000b). These habitats vary in pH (4.75–10.03) and temperature (59.0–71.4°C), as well as other measured geochemical parameters. In submarine hydrothermal sediment from Vulcano's Baia di Levante, the observed prokaryotic community was identified by FISH as ∼30% thermophilic archaea and ∼14% thermophilic bacteria (Rusch et al. 2005).

The dominant groups were Crenarchaeota and Aquificales, although Thermococcales, Archaeoglobales, *Thermus*, *Thermotoga,* and *Bacillus* were observed as well. In sediment cores from Paleochori Bay at Milos, the abundance of archaea relative to bacteria increased with increasing depth and temperature (Sievert et al. 2000b). Archaeal taxa closely related to *Stetteria*, *Thermococcus*, *Pyrococcus*, *Staphylothermus*, *Desulfurococcus*, and *Thermodiscus* (Dando et al. 1998, and a broad diversity of eight lineages of Bacteria (dominated by the Cytophaga-Flavobacterium and Acidobacterium groups) (Sievert et al. 2000a) were identified by culturing and molecular analysis. In a crater-like basin off of Taketomi Island, Japan, a cooler (52°C) sulfide and methane rich environment was shown to support autotrophic sulfide and methane oxidizing organisms (Hirayama et al. 2007). Last, in the alkaline smectite vent fluids at Eyjafjordur, Iceland, clone libraries built from extracted DNA were dominated by Aquificales and Korarchaea (Marteinsson et al. 2001). Unlike the slightly acidic and circumneutral sites of Vulcano and Milos, neither Crenarchaeota nor Euryarchaeota are not prevalent at the Iceland location.

From a geochemical viewpoint, arguably one of the best characterized shallow-sea HVSs is that in Tutum Bay, Ambitle Island, Papua New Guinea (Pichler and Dix 1996; Pichler and Veizer 1999; Pichler et al. 1999, 2000; Price and Pichler 2005). However, the prokaryotic communities there have not been documented. Tutum Bay features several high flux vents (up to 400 L/min), surrounded by diffuse flow fields. Of particular interest are the effects of elevated arsenic concentrations on the surrounding marine ecosystem (Pichler et al. 2006). The high temperature (89–98°C), circumneutral fluids discharge ∼1.5 kg of arsenic/day into a small (∼50 × 100 m) bay. Arsenate (As^V) is co-precipitated with hydrous ferric oxides (HFOs) which coat surrounding sediments and coral; these HFO precipitates contain up to 7 wt% arsenate (Pichler and Veizer 1999; Pichler et al. 1999b). It has been shown in the acidic (pH 3.1) continental hot springs of Norris Geyser Basin (Yellowstone National Park, USA), that microbially mediated arsenite (As^{III}) oxidation can be rapid in hydrothermal systems. In fact, the fastest arsenic oxidation rate in any natural aquatic system was recorded in one Norris hot spring (NHSP 106). There, a variety of bacteria and archaea have been identified, including species of uncultured Crenarchaeota, *Hydrogenobacter*, *Desulfurella*, Thermoplasmatales (NHSP106) (Jackson et al. 2001), *Acidimicrobium*, *Thiomonas*, *Metallosphaera*, and *Marinithermus* (NHSP35) (Inskeep et al. 2004). Unlike the Tutum Bay hydrothermal system, the thermophile population at Norris is influenced (and inhibited) by the presence of sulfide in the hot spring fluids. In the Norris Geyser Basin springs, arsenite oxidation, ferrihydrite precipitation, and biogenesis of arsenate-rich HFO mats appear to be directly influenced by the presence of sulfide and its consumption by archaeal and bacterial taxa (Donahoe-Christiansen et al. 2004; Inskeep et al. 2004; Macur et al. 2004).

As noted, the Tutum Bay shallow-sea ecosystem is characterized by ferrous iron- and arsenite-rich vent fluids and HFO precipitation, but the role of microorganisms in the related redox processes is unknown. With exception of the previously mentioned works, iron and arsenic biogeochemistry is poorly understood in shallow marine hydrothermal environments, and indeed in most environments; we refer the reader to several recent review papers covering these areas (Edawrds et al. 2004, 2005; Stolz et al. 2010). As part of a first-order investigation of biogeochemical processes in the Tutum Bay hydrothermal system, here we synthesize fundamental geochemical and microbial community structure data sets. A transect beginning at a single vent (Vent 4, Figure 1) and extending to 60 m was established (transect 4A), and the prokaryotic community in the heated sediment 2.5 m from the focused vent source was investigated. Sediment cores were retrieved, and two depth horizons were chosen for prokaryotic community analysis. Geochemistry of pore fluids from 2.5 m and along a transect extending to 60 m from the vent source was analyzed. This integration of datasets allows interpretation of the subsurface fluid flow and environmental context for the discovered prokaryotic communities, laying the groundwork for future studies of ecological function and biogeochemical cycling.

METHODS

The Tutum Bay sample site (previously described in Pichler and Dix 1996; Pichler 1999a 1999b; Price and Pichler 2005; Price et al. 2007), offshore of Ambitle Island, Papua New Guinea, is the focus of a collaborative effort to investigate the effect of arsenic on a marine ecosystem. Companion studies regarding the geochemistry, macrofauna, and meiofauna along two transects (A and B) originating at one vent source (Vent 4) have been published or are in progress (Price et al. 2000, 2007; Akerman and Amend 2008; Karlen and Garey 2005; Karlen et al. 2007a, 2007b; Price and Pichler 2005; McCloskey 2009). The Tutum Bay field location was investigated during two separate cruises, in November 2003 and May 2005, during which transects A and B were established by setting in place aluminum

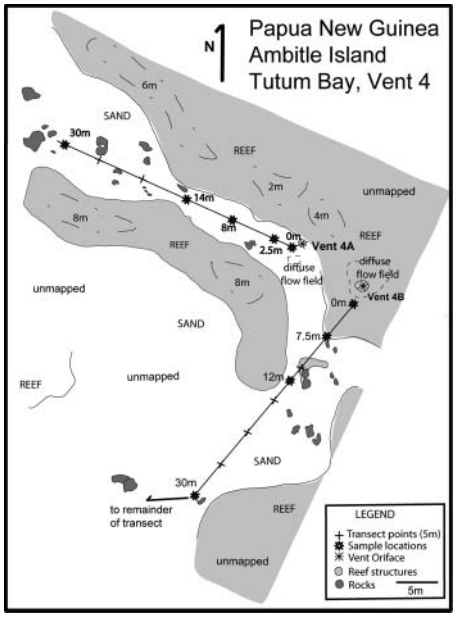

FIG. 1. Site location map of Ambitle Island, Tutum Bay sampling area as mapped in 2003. Sampling area was approximately 10m deep. Height of the surrounding reef structures from the sea bed are noted. Transect 4A extends from Vent 4A in approximately a NNW direction. Transect 4B is shown for reference to related reports. Sediment cores were collected at the 2.5 m site on transect 4A.

stakes connected by a metered rope. This communication is concerned with transect "4A" (Figure 1), which was established in an area of soft, sandy sediment between reef structures in a NNW direction from the vent source.

Sample Collection

Sediment cores were taken in November 2003 and May 2005 from the shallow-sea Vent 4 area in Tutum Bay. The cores were retrieved by SCUBA, using a 1 m length of PVC pipe (6 cm diameter), at 2.5 m along transect 4A, in an area of soft sediment (Figure 1). As seen in Figure 1, Vent 4 is surrounded by coral reef and hard rubble, permitting the nearest sediment coring at a distance of 2.5 m along trasect 4A. The core barrel was manually driven 90 cm into the sediment, capped on top, retracted, quickly capped at the bottom, and immediately returned upright to the surface, preserving the stratification. On deck of the ship, the pipe was cut lengthwise to expose the sediment. The cores were photographed, and the center of the split core was subsampled at apparent redox boundaries as indicated by bands of HFOs in the sediment. Two subsamples for DNA extraction were taken from the 2003 core with a sterile spatula (avoiding contact with the PVC pipe) and immediately frozen (-20°C) until analysis. Subsamples for analysis of trace elements/metals were collected in small vials, acidified, and stored at room temperature until analysis.

Vent fluid and pore fluid samples along transect 4A were obtained in November 2003 and May 2005 as previously described (Price and Pichler 2005). Briefly, pore water profiles to a depth of $\sim$1 m were collected through Teflon® tubing in an aluminum pipe with screened openings every 10 cm. Water was drawn slowly and simultaneously from up to 6 horizons at a time.

The sample naming convention used for both core sediment and pore fluid samples is as follows: vent/transect/year-distance (m)-depth (cm). Thus sample name "4A05-2.5-10" indicates the sample was collected in the Vent 4 area, on transect A, in 2005, 2.5 m from the source of Vent 4A, at 10 cm depth.

Geochemistry

Pore fluid samples collected in 2005 were analyzed on board immediately after collection for redox sensitive species (ferrous iron, ammonia, sulfide, nitrate, nitrite, dissolved oxygen), phosphate, and silica using a portable spectrophotometer (HACH Co., CO, USA). (For specifics pertaining to these analyses, see Amend et al. 2003.) Alkalinity was determined by titration (HACH), and pH was measured with a Myron-L pH meter with temperature compensation. Samples of pore and vent fluids were collected in both 2003 and 2005, preserved by filtration and acidification if necessary (e.g., for cation and arsenic abundance and speciation), and analyzed for major anions (ion chromatography, MacCarthy-Dionex) and cations (ICP-OES) as previously described (Price and Pichler 2005; Price et al. 2007).

Analyses of arsenic abundance and speciation in sediments and fluids collected in 2003 and 2005 were performed in the Pichler lab (USF). Samples were collected by push cores, appropriately preserved in the field (by filtration and acidification), and analyzed as specified in Price and Pichler (2005). Analyses of trace elements and metals were performed as described previously (Price and Pichler 2005; Price et al. 2007). Briefly, arsenic and iron concentrations were measured following acid digestion by ICP-MS and atomic fluorescence spectrometry (AFS). Speciation of arsenic in solids was also determined by energy dispersive X-ray (EDX).

DNA Extraction from Sediment Samples

Bulk environmental (genomic) DNA was extracted on site from subsamples of core 4A03-2.5. Here, the focus is on two horizons: "Horizon 2", sampled at 12–16 cm depth, and "Horizon 4", at 28–32 cm depth. Two methods were applied to each sediment sample, because different DNA extraction methods have different recovery efficiencies (Hugenholtz et al. 1998; Miller et al. 1999): bead-beating using the Bio101 extraction kit (Q-BIOgene's FastDNA Spin Kit for Soil), and a general chemical extraction (Qiagen), in each case adapting the manufacturer's instructions concerning size/mass of extraction material to the sample characteristics. Extracted DNA was amplified with primer pairs 21F-1391R (targeting Archaea) and 27F-1492R (targeting Bacteria) (Lane 1991) using a Hybaid PCR Express thermalcycler, as described previously (Meyer-Dombard et al. 2005).

Molecular Cloning of Amplified Community DNA

PCR products from the two extractions were combined and cloned using the Invitrogen TOPO-TA cloning kit (cat# K4500-01). This resulted in four libraries, one archaeal and one bacterial for each of the two samples. The efficacy of the DNA

extraction methods was not evaluated in this study. Between 192 and 384 white colonies were picked from each library, and the plasmids were purified using the QIAGEN miniprep kit (cat# 27106). The insert was amplified using M13F and M13R primers, and screened using short-segment sequencing, as previously described (Meyer-Dombard et al. 2005). The Tutum Bay transect 4A Horizon 2 and Horizon 4 clonal libraries were named "4AH2" and "4AH4", respectively.

Sequencing and Phylogenetic Inference

Following the initial screening of clone libraries described above, which enabled identification of unique clones, the full 16S rRNA gene was sequenced for unique clones from the transect 4A H2 (39 clones), and 4AH4 (29 clones) libraries. Clones with DNA inserts were directly sequenced using the primers 21F/958R/1391R (targeting Archaea) and 27F/907R/1492R (targeting Bacteria), as described in Meyer-Dombard et al. (2005).

Contiguous sequences were assembled using SEQUENCHER (v4.1.4, Gene Codes Corporation), submitted to GenBank (accession numbers EF100619- EF100651), and compared to the NCBI (National Center for Biotechnology Information) database using a BLASTn search against the nr/nt database (Altschul et al. 1997) to find closest relatives. The software BioEdit (v5.0.9; http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was used to align approximately 1200-1400 nucleotide bases, which were then adjusted manually using the predicted secondary structure of the molecule as a template. Chimeric analyses were largely performed by careful alignment comparisons, but the ChimeraCheck (Cole et al. 2003) and Bellerophon (Huber et al. 2004) software were also used as guidelines. Phylogenetic inference of homologous positions was performed using neighbor joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) with the software PAUP (version 4b.10; Sinhauer Associates, Sutherland, Mass.), as reported in Meyer-Dombard et al. (2005). All trees were evaluated using only unambiguously aligned nucleotides and bootstrapped (100–1000 replicates) (Felsenstein 1985). Taxa not uniquely affiliated with a phylogenetic position are noted in the results. Genbank deposits are named following the convention "PNG_TB_4A2.5", indicating they are from Papua New Guinea, Tutum Bay, and further named either "H2" or "H4", indicating the sediment horizon from which they originated.

Microscopy

Sediment samples (collected in 2 ml tubes) from core 4A03-2.5 were preserved with PBS-buffered formalin (4%) for at least 2 h before storage in 1:1 PBS:EtOH at −20°C (Rusch and Amend 2004). Cells were dislodged from sediment surfaces using a mild ultrasonic treatment (2 min, pulsed), and suspended in a known volume of PBS:EtOH. Samples of pore fluid for microscopy, as close to the vertical location of the sediment samples as possible, were preserved by adding paraformaldehyde (5% total conc.). Sediment preparations and fluids were concentrated on polycarbonate filter membranes (0.2 μm pores), air dried, and stored at -20°C until hybridization. Whole cell counts using DAPI (4′-6-diamidino-2-phenylindole) staining, and fluorescence in situ hybridizations (FISH) targeting Archaea (probes Arch917 + Arch344), Bacteria (probes Eub338 + Aqui1197 + hAqui1045), and the orders Thermotogales (probe Ttoga660) and Aquificales (Aqui1197 + hAqui1045) were conducted as outlined in Rusch and Amend (2004) and examined by epifluorescence microscopy at 1300x magnification (Zeiss Axioskop2). In FISH enumeration, the unspecific-stained background signal (using the probe NON338) was <5% of the total DAPI stained cells for all samples analyzed.

RESULTS

Core Description

The thermal sediment retrieved near the vent source was coarse-grained, altered material with high HFO content. Several potential redox boundaries featuring these oxidized deposits were visible, and these zones were subsampled. Although the sampling device was driven into the thermal sediment to 90 cm in each instance, some compression occurred.

Temperature and pH at 2.5 m and Along Transect 4A

Temperature and pH follow an irregular pattern in the first 60 m of transect 4A and within core 4A03-2.5 (Figures 2, 3). In core 4A03-2.5 at depths corresponding to the FISH analyses (shown by arrows in Figure 3) pore fluid temperature varied by 20°C, and pH varied only by 0.1 pH units. The temperature at ∼50 cm was the lowest measured within the core. In addition, "Horizon 4" (28–32 cm depth) was nearly 10 degrees cooler than "Horizon 2" (12–16 cm depth). In general, sediment temperature decreased with distance, but spiked sharply by 20 degrees 30 m away from the vent source. These findings are similar to the

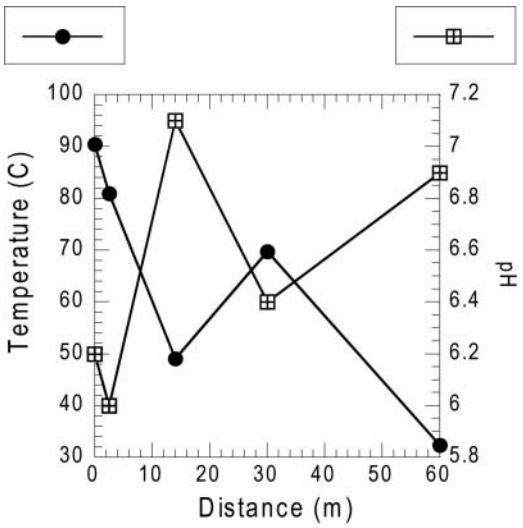

FIG. 2. Profile of temperature (filled circles) and pH (squares) with distance (measured at 10cm depth) along transect 4A.

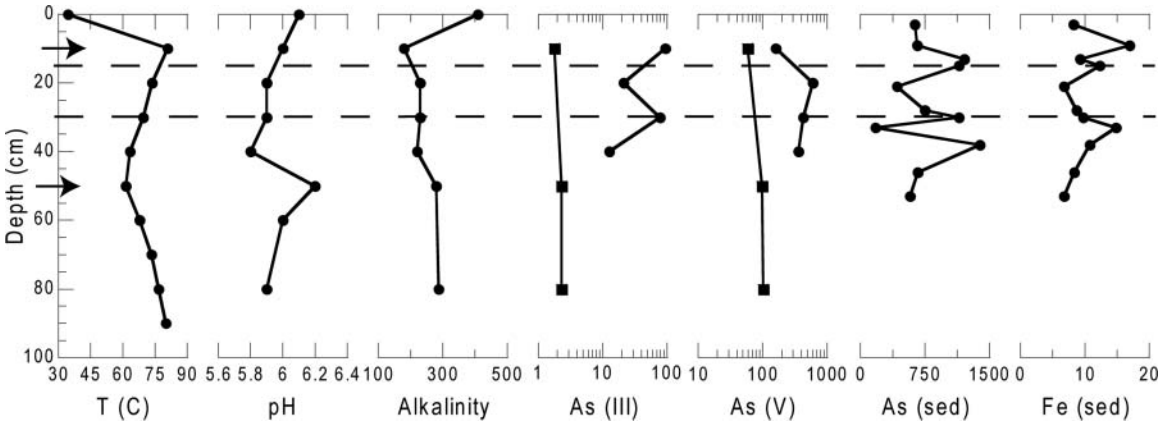

FIG. 3. Measurements of physical and chemical conditions in pore fluids and sediments in core collected 2.5m from the beginning of transect 4A. As(III), As(V) in μg/L, circles are 2003 data, squares are 2005 data; As(sed), Fe(sed) in mg/l; Alkalinity in mg/L CaCO3. Arrows indicate sample depth of FISH analyses, dashed lines show average sample depth of 16S rRNA gene surveys (of compacted core sediment) where Horizon 2 is 12–16 cm and Horizon 4 is 28–32 cm deep.

data reported for temperature and pH along transect 4B (Price and Pichler 2005; Price et al. 2007). It was found on transect 4B that the expected drop in temperature and rise in pH with distance from the vent did not occur until 50 m away from the vent source, and there were spikes in temperature as far away from the vent as 75 m.

Sediment and Fluid Geochemistry of Transect 4A

Analysis of major ion chemistry in pore fluids along transect 4A at 10 cm depth (Tables 1 and 2) shows a decrease in alkalinity with distance away from the vent source, from 500 mg/L at the vent to <200 mg/L by 2.5 m and to <100 mg/L by 30 m. Total iron concentrations were very low compared to the vent fluid. Concentrations of calcium, potassium, sodium, sulfate, chloride, and magnesium were depleted at the vent source, relative to seawater, but approach seawater values by 2.5 m on the transect. Total arsenic concentrations in the sediment on transect 4A vary from 175 ppm to >1200 ppm, while concentrations in pore fluid were much more dilute (50–620 ppb), as also demonstrated by earlier studies along transect 4B (Price and Pichler 2005). However, even at 30 m, As values were still more than 30× the background seawater value.

Sediment and Fluid Geochemistry in Core at 2.5 m

Major ions showed variation with depth over time (Tables 1 and 2). Redox sensitive ions varied in concentration within the core; sulfide and phosphate increased, nitrate and nitrite decreased, and dissolved oxygen was variable with depth. Total iron was not measurable in the pore fluids with depth in the core. Potassium and calcium concentrations resembled seawater at the shallowest depths. Similar to temperature and pH, Figure 3 shows that no apparent trend existed in the concentrations of arsenic in the sediments or pore water at 4A-2.5. In general, as shown by Price and Pichler (2005), arsenic was most concentrated in areas of heavy HFO precipitation. Concentrations of arsenic in the sediments at 12–16 cm depth, and 28–32 cm depth (dashed lines, Figure 3), which are the horizons sampled for DNA extraction, were among the highest concentrations measured in the core and were around twice that measured at 10 cm and 50 cm, the locations of the pore fluid FISH analysis (1150–1210 ppm vs. 576–663 ppm, respectively).

Cell Counts in Pore Fluids

Microbial cell counts in pore fluids from the 10 cm and 50 cm depths of core 4A03-2.5 were 9.8×10^4 cells/ml and 8.3×10^4 cells/ml, respectively. FISH analyses showed that archaea made up 19% and 16%, and bacteria 81% and 83.5% of the DAPI-stained cells in the 10 cm and 50 cm samples, respectively (~0.5% of the 50 cm sample did not stain with either probe set) (Figure 4a). FISH probes specific for the thermophilic bacterial orders Thermotogales and Aquificales were also applied to the pore fluid samples. Hybridizations with the Aquificales probe set (Aqui1197 + hAqui1045) were not successful, but a 16S rRNA gene survey (see later) revealed the presence of close relatives to the Aquificales. Approximately 9% of the total cells (and 12% of the bacterial cells) in the 10 cm sample hybridized with the Thermotogales probe; no cells hybridized with the Thermotogales probe in the 50 cm sample (Figure 4a). Whole cell DAPI counts and FISH analyses on core sediments were not successful. Heavy particulate load of HFO precipitates obscured most of the stained cells on the filters, and prevented accurate cell counts. Sonication of the sediment resulted in flocculent particulates that further masked the cells.

16S rRNA Gene Survey

All clones with nearly complete 16S rRNA gene sequences are listed in Table 3, along with their phylogenetic affiliations, the closest GenBank match (by BLAST analysis), and percentage similarity to the GenBank data. Rarefaction curves for each of the Horizon 2 libraries (using between 192–384 clones, data not shown) indicate that the bacterial and archaeal community diversity at the genus level saturated after the analysis of 30 and

TABLE 1
Field data collected from pore fluids along transect 4A and with depth in sediment cores (2003 and 2005 data)

Sample number	year	distance m	depth cm	Temp °C	pH	Salinity	Alk. mg/L	HCO_3^- mg/L	Fe^{+2} mg/L	NH_4^+ mg/L	S^{-2} μg/L	NO_3^- mg/L	NO_2^- mg/L	DO mg/L	PO_4^{-3} mg/L
4A03-0-10	Nov 2003	0	10	90.5	6.2	—	500.2	610.0	—	—	—	—	—	—	—
4A03-2.5-0	Nov 2003	2.5	0	34.6	6.1	—	410.0	500.2	—	—	—	—	—	—	—
4A03-2.5-10	Nov 2003	2.5	10	81.0	6.0	—	180.0	439.2	—	—	—	—	—	—	—
4A05-2.5-10	May 2005	2.5	10	N/A	6.1	32.0	144.3	176.0	0.02	UR	3.0	8.4	0.02	4.9	0.27
4A03-2.5-20	Nov 2003	2.5	20	73.6	5.9	—	230.0	561.2	—	—	—	—	—	—	—
4A03-2.5-30	Nov 2003	2.5	30	69.5	5.9	—	230.0	561.0	—	—	—	—	—	—	—
4A03-2.5-40	Nov 2003	2.5	40	63.5	5.8	—	220.0	536.8	—	—	—	—	—	—	—
4A05-2.5-50	May 2005	2.5	50	61.6	6.2	30.0	280.1	341.6	0.02	UR	4.0	7.7	0.02	3.2	0.42
4A05-2.5-80	May 2005	2.5	80	76.7	5.9	23.0	288.1	351.4	UR	UR	8.0	6.7	0.013	5.1	0.55
4A03-30-10	Nov 2003	30	10	69.8	6.4	—	92.0	112.2	—	—	—	—	—	—	—
4A03-60-10	Nov 2003	60	10	32.3	6.9	—	84.0	102.5	—	—	—	—	—	—	—
4A-VW	Nov 2003	0	N/A	92.7	6.2	—	532.2	649.0	—	—	—	—	—	—	—
4A-VW	May 2005	0	N/A	91.9	6.1	1.00	588.00	717.36	0.97	0.09	0	0.15	0.01	1.10	2.06
SW	Nov 2003	N/A	N/A	28.0	8.0	37.90	118.90	145.00	—	—	—	—	—	—	—
SW	May 2005	N/A	N/A	—	—	—	114.52	139.66	—	—	2.0	8.10	—	4.00	0.03

"UR" = under range, "–" = not analyzed.

TABLE 2
Major ion chemistry from pore fluids along transect 4A and with depth in sediment cores (2003 and 2005 data)

Sample number	distance m	depth cm	As, tot. μg/L	As(III) μg/L	As(V) μg/L	Si mg/L	Ca^{+2} mg/L	Fe,(tot) mg/L	K^{+} mg/L	Mg^{+2} mg/L	Mn^{+2} mg/L	Na^{+} g/L	Cl^{-} g/L	SO_4^{-2} g/L
4A03-0-10	0	10	1031.2	1031.2	0.0	85.2	223.5	0.5	162.0	230.0	0.2	2.525	2.999	1.037
4A03-2.5-0	2.5	0	—	—	—	4.0	420.0	—	394.0	1290.0	—	10.600	16.261	2.270
4A03-2.5-10	2.5	10	255.5	95.2	160.3	61.5	423.5	0.0	258.0	709.5	0.5	5.650	7.649	1.612
4A05-2.5-10	2.5	10	60.2	1.8	58.4	8.7	403.0	—	374.0	1220.0	—	9.950	17.846	2.624
4A03-2.5-20	2.5	20	620.9	21.3	599.6	68.2	396.0	0.0	243.0	592.0	0.5	4.700	—	—
4A03-2.5-30	2.5	30	507.8	77.3	430.5	63.7	378.0	0.0	253.0	678.0	0.5	5.250	8.839	1.718
4A03-2.5-40	2.5	40	371.1	12.8	358.4	67.8	526.0	0.0	282.0	864.0	0.5	6.440	—	—
4A05-2.5-50	2.5	50	100.0	2.3	97.7	12.5	391.0	—	350.0	1130.0	—	9.140	17.232	2.585
4A05-2.5-80	2.5	80	104.8	1.4	103.4	19.1	304.0	—	287.0	834.0	0.1	7.190	12.074	2.117
4A03-30-10	30	10	167.3	1.9	165.5	6.2	412.0	0.0	392.0	1300.0	0.0	10.700	19.763	2.710
4A03-60-10	60	10	—	—	—	3.8	415.0	—	400.0	1310.0	—	10.700	20.113	2.769
4A-VW	0	N/A	891.9	891.9	0.0	92.8	220.8	1.1	142.4	129.8	0.4	1.805	1.878	1.112
4A-VW	0	N/A	878.70	—	—	100.9	209.22	0.85	125.56	31.56	0.32	1.124	1.181	0/974
SW	N/A	N/A	—	—	—	1.60	434.00	—	385.00	1270.00	—	9.960	20.457	2.829
SW	N/A	N/A	—	2.57	2.29	—	416.00	—	394.00	1289.00	—	10.700	194.55	2.666

"UR" = under range, "–" = not analyzed.

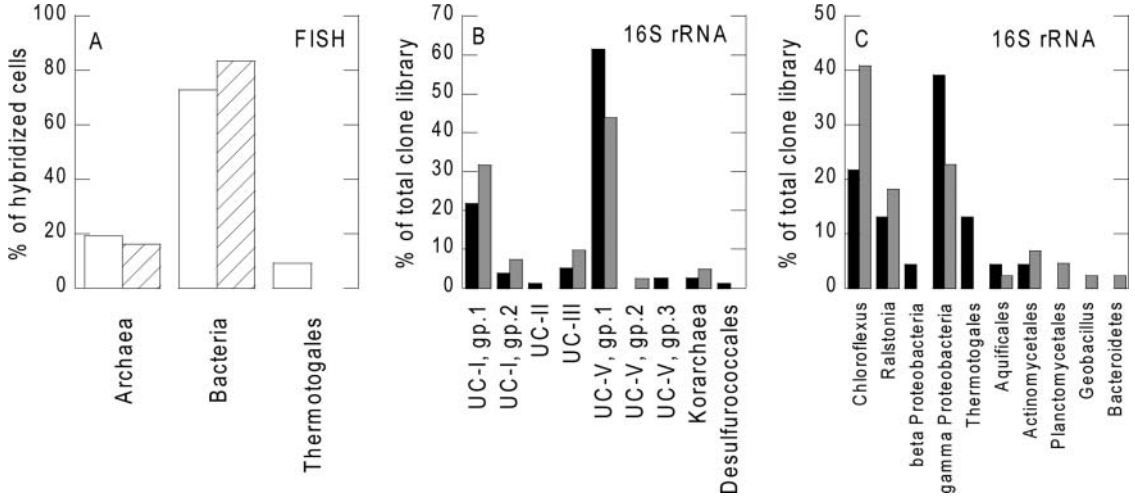

FIG. 4. Results of FISH and 16S rRNA gene survey in core 4A03-2.5. (A) FISH analyses of pore waters, 10 cm (white) and 50 cm (striped) fractions. Bars represent the percentage of the total number of hybridized cells counted for each sample. (B/C) Diversity and distribution of clones within archaeal (B) and bacterial (C) clone libraries for Horizon 2 (black) and Horizon 4 (grey) sediment samples. Bars indicate the percentages of each taxon in the total respective clone library, as determined by screens of partial (∼700 bp) 16S rRNA gene sequences. Horizon 2 = 12–16 cm; Horizon 4 = 28–32 cm depth.

60 clones, respectively; the H4 libraries maximized after the analysis of 60 bacterial and 30 archaeal clones.

Phylogenetic analyses showing inferred positions of H2 and H4 clones (in bold type), relative to other sequences deposited in GenBank, are shown in Figure 5 (archaeal analysis), and Figures 6–8 (bacterial analyses). These figures show the maximum parsimony analyses, constructed with near full length 16S rRNA gene sequences, which were in close agreement with the neighbor-joining and maximum-likelihood tests. Uncultured Crenarchaeota in Figure 5 include groups variably labeled in other reports as the "Terrestrial Miscellaneous Crenarchaeotic Group", "Miscellaneous Crenarchaeotic Group", "Hyperthermophilic Crenarchaea", and the "Marine Hydrothermal Vent Groups" (Inagaki et al. 2003; Takai and Horikoshi 1999; Takai et al. 2001), but not groups typically labeled as "Marine Crenarchaeal Groups".

The distribution and diversity of clones representing unique taxa (as determined by phylogenetic analysis) are given in Figures 4(b,c) as percentages of the total number of clones screened in each library. Histograms represent 4AH2 and 4AH4 archaeal libraries (Figure 4b) and bacterial libraries (Figure 4c). Note that this representation reflects the proportions of each unique clonal group within the clone library, but not necessarily the abundance within the core sediments. The sediment microbial communities at the two horizons share many members, but in very different proportions within the clone libraries. Figure 4b shows that the archaeal clone libraries from both horizons were composed of similar "genus" level groups (92% similarity cutoff) in differing proportions in core 4A03-2.5. In both horizons, the archaeal diversity is dominated by uncultured Crenarchaeota (UC) in Group V (∼45–60%) and Group I (∼20–30%).

Less than 10%, and generally <5%, fall among UC II, UC III, the Korarchaeota, and the Desulfurococcales. Notably, Euryarchaeota-like sequences were absent from both the H2 and H4 clone libraries. Among the bacteria (Figure 4c), H2 features predominantly γ-Proteobacteria (39%), Chloroflexus (22%), Ralstonia (13%), Thermotogales (13%), and β-Proteobacteria, Aquificales, and Actinomycetales (each <5%). H4 is dominated by Chloroflexus (41%), followed by γ-Proteobacteria (23%), Ralstonia (18%), Actinomycetales (7%), and Planctomycetales, Aquificales, Geobacillus, and Bacteroidetes (each <5%).

DISCUSSION

Fluid Mixing in the Subsurface

The temperature and pH of the pore fluids horizontally and vertically along transect 4A (Figures 2, 3) are directly related to the diffusion of vent fluid through the sediments surrounding the vent source, and it is seen that the diffusion of vent fluid is not a simple function of distance or depth. Seawater mixing in pore fluids was variable at the 2.5 m location with time (Figure 9). In both the 2003 and 2005 sediment cores, the pore fluids at the surface of the core (0 cm) were more similar to seawater, and the vent fluid signature (as noted by the decrease in Mg concentration) increased with depth in the sediment. Several major ions measured in pore fluids 2.5 m away from the vent source, such as magnesium, sodium, and chloride, and silica (SiO_2) showed variation in the degree of seawater mixing at depth over time (Tables 1 and 2).

In 2003, these ions reached a concentration intermediate between vent and seawater values and were variable with depth, while in 2005, a larger seawater component was evident. Further, the profile of arsenic concentration likely indicates a complex and rapidly changing redox profile within the sediments of Tutum Bay. For example, As concentrations in pore fluids were lower in 2005 but more consistent with depth, while

TABLE 3
Record of 16S rRNA gene clones and affiliated taxa. Full clonal names all begin with "PNG_TB_4A2.5", as reported to NCBI

Clone# (type representative)	Fig #	Phylogenetic Group	Closest GenBank match (% similarity)
PNG 4A H2 A34	5	Desulfurococcales	cl. SUBT-9 (AF361213) 95%
PNG 4A H2 A57	5	UC-I, group 1	cl. YNP ObP A97 (DQ243761) 93%
PNG 4A H4 A55	5		cl. YNP ObP A136 (DQ243747) 92%
PNG 4A H2 A33	5	UC-I, group 2	cl. YNP ObP A97 (DQ243761) 93%
PNG 4A H4 A20	5		cl. IAN1-16 (AB175577) 88%
PNG 4A H2 A60	5	UC-II,	cl. VulcPIw.170 (DQ300328) 98%
PNG 4A H2 A3	5	UC-III	cl. FnvA66 (AB213076) 92%
PNG 4A H2 A64	5		cl. FnvA66 (AB213076) 92%
PNG 4A H4 A24	5		cl. FnvA66 (AB213076) 92%
PNG 4A H2 A51	5	UC-V, group 1	cl. SUBT-11 (AF361214) 97%
PNG 4A H4 A52	5		cl. SUBT-11 (AF361214) 97%
PNG 4A H4 A34	5	UC-V, group 2	cl. 10-H-08 (AB201309) 92%
	5		
PNG 4A H2 A46	5	UC-V, group 3	cl. FnvA94 (AB213086) 91%
PNG 4A H2 A74	5		cl. FnvA94 (AB213086) 91%
PNG 4A H2 A8	5	Korarchaeota	cl. pOWA133 (AB007303) 95%
PNG 4A H4 A53	5		cl. pOWA133 (AB007303) 95%
PNG 4A H2 B34	6	Actinomycetales	*Propionibacterium* sp. (AY642051) 99%
PNG 4A H4 B69	6		*Rhodococcus facians* (Y11196) 99%
PNG 4A H2 B17	6	Chloroflexus	cl. IBC2-9 (AB175559) 92%
PNG 4A H4 B146	6		cl. IBC2-9 (AB175559) 92%
PNG 4A H2 B29	7	B Proteobacteria	cl. pHAuB-34 (AB072717) 98%
PNG 4A H2 B26	7	γ Proteobacteria	cl. G73 (AF407710) 88%
PNG 4A H4 B40	7		cl. G73 (AF407710) 88%
PNG 4A H4 B97	7		cl. G73 (AF407710) 88%
PNG 4A H4 B32	6	Bacteroidetes	cl. CH4 1 BAC (AY304377) 99%
PNG 4A H4 B157	6	Firmicutes	*Geobacillus tepidamans* (AY563003) 99%
PNG 4A H4 B56	7	Planctomycetales	cl. OPB17 (AF027057) 90%
PNG 4A H2 B11	8	Aquificales	*Hydrogenobacter* sp. (AJ320215) 96%
PNG 4A H4 B47	8		*Hydrogenobacter* sp. (AJ320215) 96%
PNG 4A H2 B67	8	Thermotogales	cl. EM 3 (U05660) 93%
PNG 4A H2 B114	8		cl. EM 3 (U05660) 93%
PNG 4A H2 B91	—	Ralstonia	*Ralstonia pickettii* (AY741342) 100%
PNG 4A H4 B190			cl. PCF29-(HA9) (AY540766) 99%
PNG 4A H2 B2	—	Misc. γ Proteobact.	*Pseudomonas* sp. (AY269867) 99%
PNG 4A H4 B12			*Pseudomonas* sp. (AY269867) 99%

concentrations were higher and highly variable in 2003. Figures 3 and 9 may also reveal differences in precipitation between 2003, and 2005 expeditions. As the mixing line in Figure 9 shows, 2005 fluids were likely diluted with seawater close to the sediment surface. Pore fluid composition in 2003 was less diluted by seawater but altered by a third component of intermediate composition, such as meteoric water. Microbial communities living deeper within the sediments are likely subjected to an environment more dominated by vent fluid than seawater. Collectively, the results indicate that the path of diffusion and mixing with local seawater was highly variable within these heated marine sediments over time, depth, and distance.

Phylogenetic Analysis and Comparison of Sediment Horizons

Members of the archaeal communities of horizons 2 and 4 were similar, but differ in proportion in the 16S rRNA gene

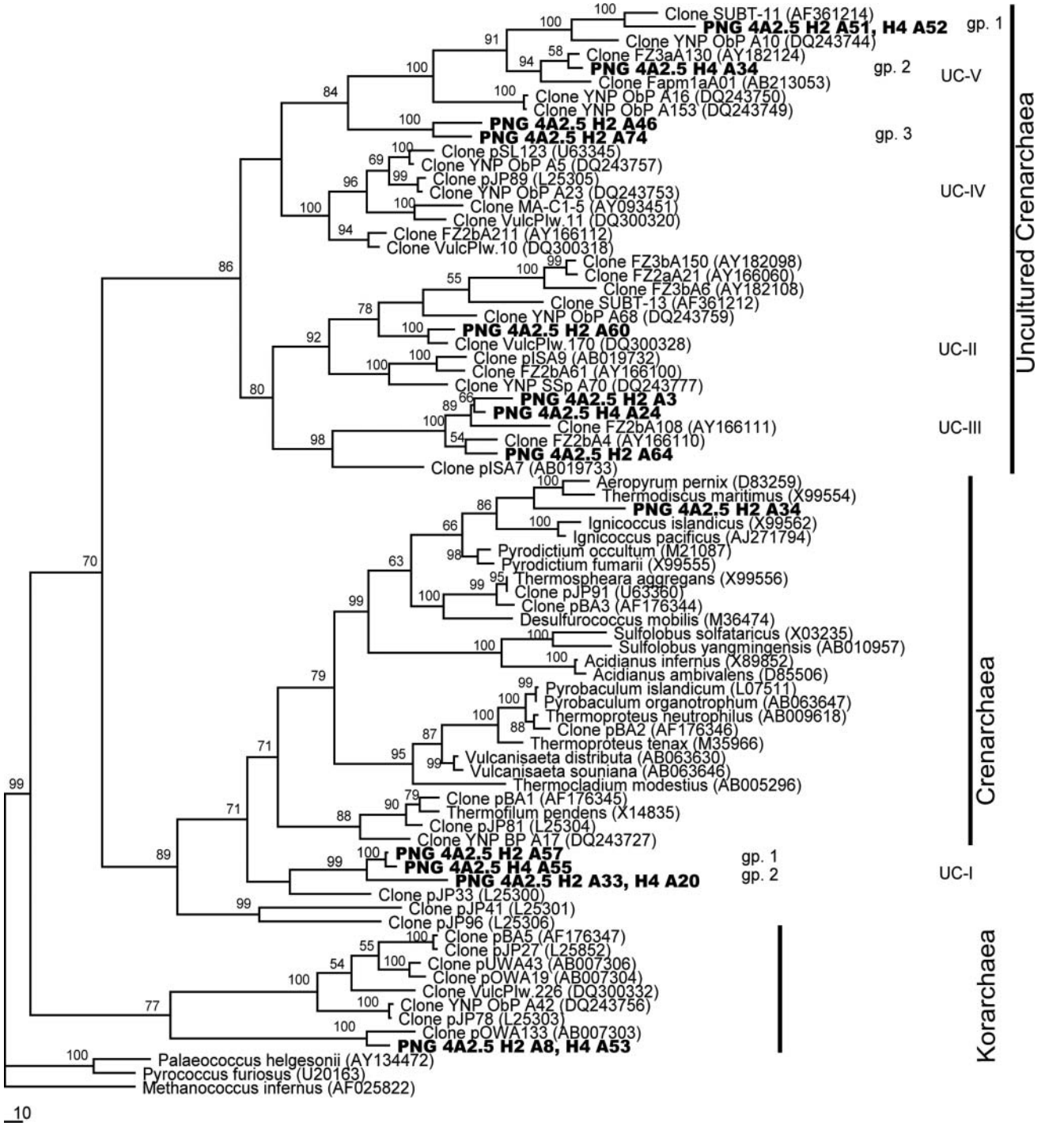

FIG. 5. Maximum parsimony phylogenetic analysis from 16S rRNA gene sequences of the Crenarchaeota. Bootstrap values indicate 100–1000 parsimony replicates. Sequences from Horizons 2 and 4 clones are in bold type. The uncultured Crenarchaeota (UC) labeling system in is consistent with that used in Meyer-Dombard et al. 2005.

clone libraries (Figure 4). The "uncultured" Crenarchaea are dominant within the Tutum Bay archaeal clone libraries. Major contributors to both clone libraries include the Uncultured Crenarchaeal UC-I and UC-V clades, with more minor components coming from UC-II, UC-III, and the Korarchaea. Uncultured Crenarchaea UCV group 2 and UC-II are unique to Horizon 2, while UC-V group 3 is unique to Horizon 4. Both the similarity in community composition between the two sediment horizons,

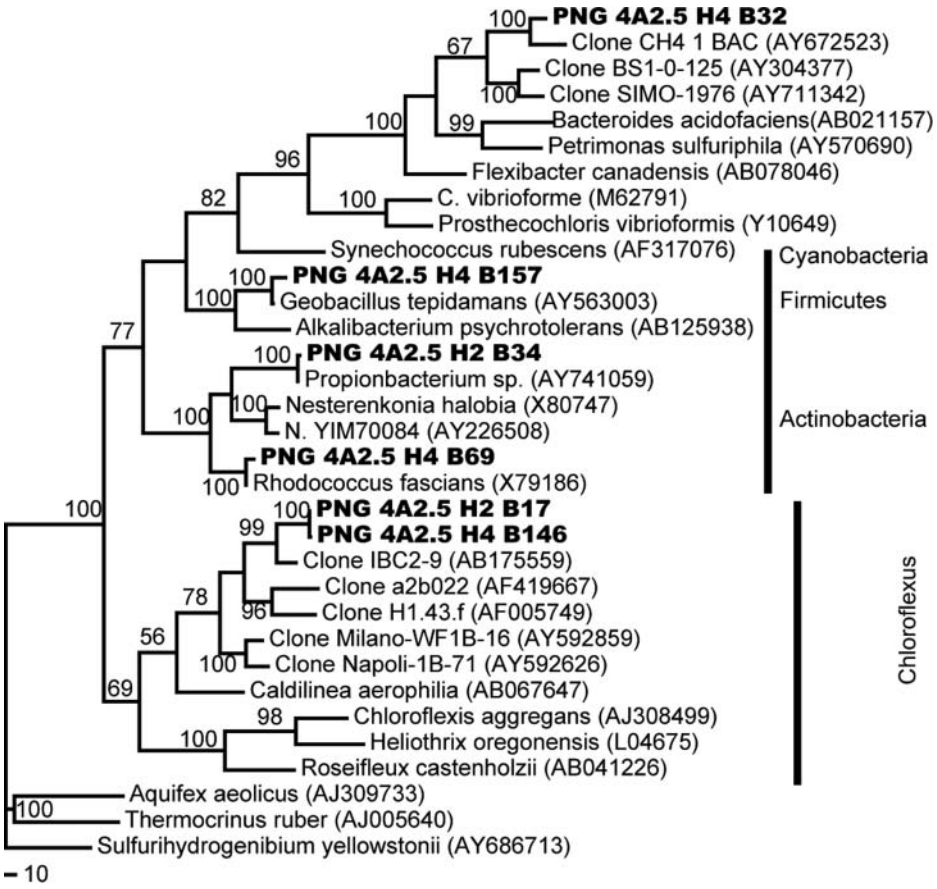

FIG. 6. Maximum parsimony phylogenetic analysis from 16S rRNA gene sequences of bacterial groups Bacteroidetes, Chloroflexus and the "gram positive" Bacteria. Bootstrap values indicate 100–1000 parsimony replicates. Sequences from Horizons 2 and 4 clones are in bold type.

and the smaller differences between them, may be reflective of the variable fluid mixing with depth as discussed above. It is likely that some species within the Uncultured Crenarchaea are more sensitive to temperature, pH, dissolved oxygen, and a host of other environmental parameters that are in constant flux in this system.

For example, previous studies have found organisms represented by UC-I and UC-V to be tolerant of a range of environmental conditions, while UC-II and UC-III may stricter environmental requirements (Meyer-Dombard et al. 2005; Spear et al. 2005). Because we suspect that the environmental conditions in the subsurface at the Tutum Bay venting area are highly variable over time, as demonstrated by data in Figures 2, 3, and 9, defining niches for Uncultured Crenarchaea in this location would be speculative.

The crenarchaeal clones affiliate closely to other 16S rRNA gene clones from a variety of marine and terrestrial hydrothermal systems (Figure 5), including the Iheya North and Juan de Fuca hydrothermal fields (Nakagawa et al. 2005; Schrenk et al. 2003; Takai and Horikoshi, 1999), Tachibana Bay shallow marine vents (Takai and Sako 1999), terrestrial systems in Yellowstone, Iceland, and Italy (Barns et al. 1994; Marteinsson et al. 2001a; Meyer-Dombard et al. 2005; Rogers and Amend 2005), as well as environments in other extreme systems (Nunoura et al. 2005; Reed et al. 2002). Clones from the Horizon 2 library affiliating with UC-V group III are unique from all other sequences analyzed and may represent novel Crenarchaea. Tutum Bay korarchaeal clones are closely related (95%) to the clone pOWA 133 from the shallow marine vent field in Tachibana Bay, Japan (Takai and Sako 1999).

Korarchaeal 16S rRNA gene signatures have been detected from samples in many hydrothermal settings; compared in Figure 5 are clones from terrestrial locations in Yellowstone, Japan, and Italy (Barms et al. 1994; Meyer-Dombard et al. 2005; Reysenbach et al. 2000; Rogers and Amend 2005; Takai and Sako 1999), and shallow submarine vent water (Takai and Sako 1999). Sequences of most Tutum Bay archaeal clones differ substantially from sequences of their nearest neighbors ($\leq$95% similarity). Thus, it appears that the archaeal 16S rRNA gene sequences recovered from the sediments on transect 4A in Tutum Bay are unique among those known to date.

Bacteria in the Horizon 2 and 4 sediments are diverse, representing eleven distinct order-level groups. In comparison, there are six to eight archaeal "orders" (based on phylogenetic

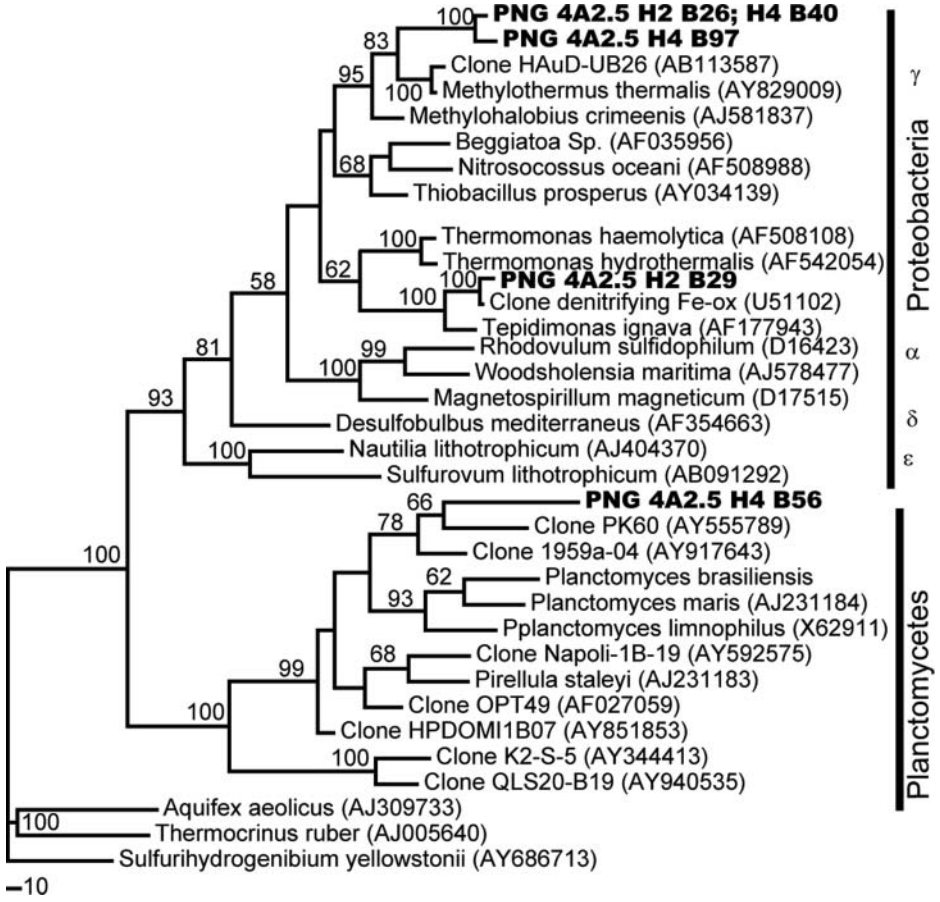

FIG. 7. Maximum parsimony phylogenetic analysis from 16S rRNA gene sequences of 5 subclasses of the Proteobacteria, and the Planctomycetes. Bootstrap values indicate 100–1000 parsimony replicates. Sequences from Horizons 2 and 4 clones are in bold type.

assignment in Figure 5) in the core sediments. As seen in Figure 4, several bacterial groups were found in both horizons (Chloroflexus, Ralstonia, gamma Proteobacteria, Aquificales, and Actinomycetales), however, other groups were unique to Horizon 2 (beta Proteobacteria and Thermotogales) or Horizon 4 (Planctomycetales, Geobacillus, and Bacteroidetes).

Strictly thermophilic Bacteria (Aquificales and Thermotogales) represent a combined 17% and 2% of the total clone library sequences in Horizon 2 and Horizon 4, respectively, despite the high temperature of the sediments in the core (∼70°C, based on pore fluid data). The Aquificales were identified in the libraries of both sediment horizons, but represent only 2–4% of the total libraries. The Thermotogales, strict anaerobes in culture, were only found in the Horizon 2 library, and represent 13% of the total clone library. This agrees well with the FISH results, which indicated the presence of Thermotoga at 10 cm within pore fluid at 4A-2.5, suggesting that this group prefers the more shallow portions of the thermal sediments (Figure 4). Two Horizon 2 clones, 4AH2 B67 and B114, form a branch within the EM3 clade (Figure 8); these clones are 93% similar to each other, and to clone EM3. It should also be considered that the strictly thermophilic and hyperthermophilic members of the community may be primarily Archaea, rather than Bacteria.

Chloroflexus-like bacteria were found in both sediment horizons, representing 22% (Horizon 2) and 41% (Horizon 4) of the bacterial clonal libraries (Figure 6). Clones from Tutum Bay falling within the Chloroflexus clade (4AH2 B17 and 4AH4 B146) are only 92% similar to clone IBC2-9, from the Iheya North Field marine hydrothermal system (Nakagawa et al. 2005), and form a clade with sequences representing other uncultured Bacteria. Other members of this clade hail from various marine sediments in the Guaymas Basin, mud volcanoes in the Eastern Mediterranean, and deep subsurface paleosols (Chandler et al. 1998; Heijs et al. 2005; Teske et al. 2002). It is unknown if the Tutum Bay Chloroflexus-like relatives are photosynthesizing in these thermal environments - given the depth and temperature of the sediments sampled, these organisms are likely utilizing another metabolic scheme. Indeed, Chloroflexus relatives have also been identified from a number of deep sea and near surface environments where photosynthetic activity seems implausible (Coolen et al. 2002; Inagaki et al. 2006; Parkes et al. 2005; Teske 2006). Pure cultures of various Chloroflexus

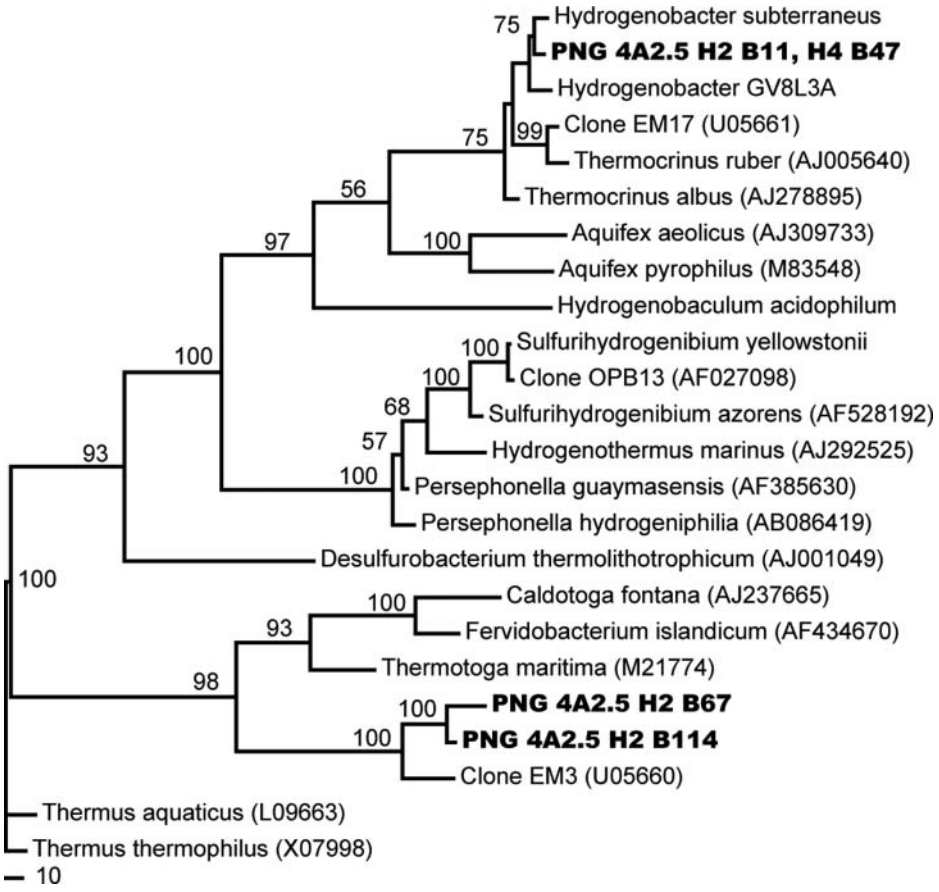

FIG. 8. Maximum parsimony phylogenetic analysis from 16S rRNA gene sequences of the thermophilic and hyperthermophilic Bacteria. Bootstrap values indicate 100–1000 parsimony replicates. Sequences from Horizons 2 and 4 clones are in bold type.

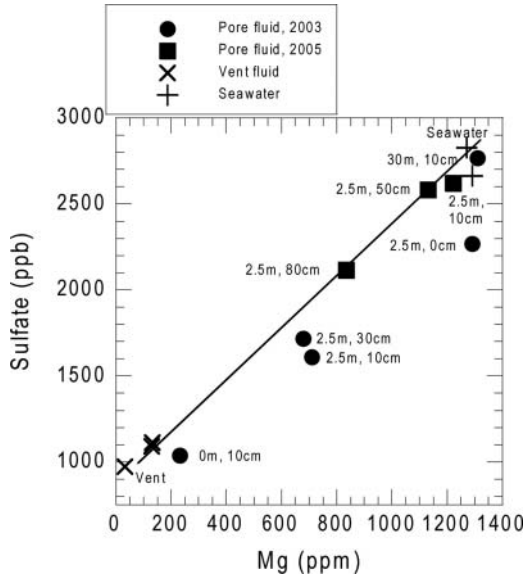

FIG. 9. Magnesium and sulfate in vent fluids (cross hatches), pore fluids (2003, circles; 2005, squares), and area seawater (plus symbol). Distance on transect and depth of pore fluid samples are given in centimeters next to the data points. Line represents a mixing line between the vent fluid and seawater endmembers.

strains have been shown to grow heterotrophically in the dark (Madigan et al. 1974; Ward et al. 1984), which suggests a possible alternative *in situ* metabolism in the Tutum Bay sediment environment.

Relatives of *Methylothermus* were also represented in the Horizon 2 and 4 bacterial libraries (Figure 7). The Tutum Bay clones occupy a novel branch within the *Methylothermus* clade; the next closely related members of the group are clone HAuD-UB26, from geothermal waters in a Japanese gold mine, and *M. thermalis* (79% similarity), isolated from hot spring sediments in Japan (Hirayama et al. 2005; Tsubota et al. 2005). In culture, *M. thermalis* is a moderate thermophile (37–67°C), capable of growth by the oxidation of methane or methanol (Tsibpta et al. 2005). Previous work has found up to 20 mmol/mol (dry gas) in the Tutum Bay vent gases; therefore, it is possible that Vent 4 *Methylothermus* relatives may be functioning as methylotrophs in this system (Pichler et al. 1999a).

The Bacteroidetes and Planctomycetes are both found in only the Horizon 4 clone library. Clone 4AH4 B32 is most closely related (99%) to another clone from the base of a white smoker chimney on the East Pacific Rise (Kormas et al. 2006). The Bacteroidetes are common in marine environments (Kirchman

2002), and while cultured members are not thermophilic, examples of Bacteroidetes have been found in clone libraries from a variety of extreme environments, including hydrothermal systems (Kanokratana et al. 2004; Kormas et al. 2006; Sievert et al. 2000a; Teske et al. 2002). The Planctomycete affiliated clone, 4AH4 B56, is possibly a novel member of this group, and is only ~75–90% similar to Planctomycetes related clones from other hot spring environments (Hugenholtz et al. 1998; Kanokratana et al. 2004). Cultured Planctomycetes and Bacteroidetes (both aerobic and anaerobic) are typically organoheterotrophic, and are known to occupy biofilms in aquatic sediments.

Interpretation of Subsurface Ecology

The goal of any ecological study is to understand the dynamic relationships between organisms, environment, and resources. This work has discovered that the majority of Archaea residing in the Tutum Bay heated sediments and all their closest relatives are uncultured; as a result analogies to metabolic functions of these Archaea based on 16S rRNA relationships are not yet possible. However, geochemical context allows calculation of energy availability and prediction of metabolic function. The mixing of Fe^{+2} and As^{+3} rich hydrothermal fluid with oxygenated seawater invites scenarios of microbial iron oxidation and precipitation, and arsenic oxidation followed by absorption onto HFOs (Pichler et al. 1999b).

Indeed, calculations of energy availability in pore fluids (10 cm depth) in the Tutum Bay venting area predict that aerobic oxidation of arsenite and ferrous iron will yield energy to microbial metabolism (Akerman and Amend 2008; Akerman et al. submitted). Iron oxidation typically occurs rapidly and abiotically at circumneutral and higher pH. However, biological mediation can increase the rate of oxidation by several orders of magnitude under certain conditions (Fortin et al. 1997).

Oxidation of diffusing thermal fluid may occur more slowly deeper in the sediments, and a perpetually shifting redox gradient provided by buoyant thermal fluid should present ample opportunities for microbial entrepreneurs. Sedimented HFOs and arsenate may be reduced in localized micro-environments (Price et al. 2007) at depth under these conditions. Our results also indicate that essential components such as fixed nitrogen, phosphate, and magnesium are measureable and variable with depth in the pore fluids. Although our 16S rRNA survey did not uncover direct evidence of organisms expected to be involved in arsenic or iron transformations, BLAST comparison revealed a 78% similarity of the Tutum Bay sequences to a clone from the 16S rRNA gene of an arsenite-oxidizing organism (Oremland et al. 2002). Investigations into the presence and expression of genes associated with arsenic and iron redox and nitrogen cycling in these sediments are underway.

Community structure analysis has revealed that a large percentage of the bacterial clones from the Tutum Bay sediment cores belong to the Chloroflexus and gamma Proteobacteria, and that the majority of cells in the pore fluids are Bacteria. As mentioned, it is unlikely that the Chloroflexus-like organisms are photosynthesizing in the subsurface, and heterotrophy is proposed as a reasonable metabolic alternative. The majority of the bacterial clones recovered are related to cultured heterotrophs, both anaerobic and aerobic, with only a few examples of close relatives to bacterial chemolithotrophs. Evidence for potential chemolithotrophic activity comes from close relatives of the genera Hydrogenobacter (hydrogen oxidizers), Tepidimonas (thiosulfate oxidizers), and Methylothermus (methane oxidizers), all of which are aerobic in pure culture. These data point to bacterial communities composed of heterotrophs and aerobic chemolithotrophs in the Tutum Bay thermal sediments. Specifics concerning the metabolic activities of Archaea in the sediments remain to be discovered.

As noted above, the geothermal fluid mixing that forms the ecological cornerstone in these thermal sediments follows no clear trend with sediment depth, or distance from the source hydrothermal input. Conditions observed between 2003 and 2005 in the same sample locations are variable [e.g., Figure 3], which is likely the result of changes in the flux of hydrothermal fluid through the system over time. Fluid flow and mixing observed or inferred within these thermal sediments likely provide a constantly changing set of redox boundaries, as shown by the variability in redox sensitive ions such as DO and sulfide, affecting the geochemical framework in the subsurface environment. The habitat suitability for aerobic and anaerobic microorganisms will likely be affected by this variable mixing between reduced thermal fluid and oxidized seawater in the subsurface. Conditions for microbial growth are clearly transitory in this geochemically driven ecosystem, and differences between the microbial communities found with depth within a single sediment core must be viewed as part of this flux.

Comparison to Other Shallow Submarine Hydrothermal Environments

Reports of microbial diversity in shallow-sea hydrothermal systems that also report physicochemical data are rare. The microbial communities in the Papua New Guinea heated sediments differ substantially from those few others that have been described. One example of a shallow-sea vent location that is directly comparable to those in Papua New Guinea is found off the island of Panaera, Italy. At one location, the bacterial communities at 0–17 cm depth were dominated by epsilon and delta Proteobacteria and Bacteroidetes, in contrast to the Chloroflexus and gamma Proteobacteria dominated bacterial communities in PNG sediments of similar depth (Amend et al. 2009).

In a study of hydrothermal sediments in Palaeochori Bay (Milos, Greece), the abundance of Archaea and Bacteria was examined by rRNA slot-blot hybridization in several sediment cores on a transect from a shallow submarine hydrothermal vent (Sievert et al. 1999, 2000b). At all depths and distances from the vent studied, these sediments were dominated by Bacteria, although Archaea gained in abundance with increasing depth (and thus, increasing temperature and pH). However, the overall rRNA abundance decreased with depth. Similar to these

findings, FISH analyses of pore fluids from the Tutum Bay hydrothermal sediments at 2.5 m away from Vent 4A (Figure 4a) indicate that Bacteria dominated in the pore fluids and total cell abundance decreased slightly with depth, from 9.8×10^4 to 8.3×10^4 cells/ml. Establishing a trend in archaeal abundance due to depth, temperature, or pH is not straightforward in Tutum Bay sediments as these conditions are variable over time (Tables 1 and 2, Figure 3).

The Tutum Bay Archaea may follow a trend similar to the Palaeochori Bay sediments, as a decrease in abundance with depth (from 19% to 16% over 40 cm) was observed with a 19.4°C drop in temperature. However, the temperature of the pore fluids increased again at greater depths for which there are no corresponding FISH data to confirm this cell abundance-temperature relationship.

CONCLUSIONS

The flux of hydrothermal fluid and resulting geochemistry of Tutum Bay shallow sea hydrothermal systems is complex, but provides niches for both heterotrophic and chemoautotrophic thermophiles. Bacteria in the core taken 2.5 m away from the venting source dominate the sediment communities and increase with depth. Bacterial 16S rRNA libraries are primarily composed of Chloroflexus and gamma Proteobacteria. Although representing a minor part of the microbial communities, decreasing with depth, the uncultured Archaea present in these sediments may play a key role in carbon and nutrient cycling. This emphasizes the need for future culturing attempts from these sediments. In addition, future culturing and targeted searches for genes relevant to nutrient and element cycling may help reveal the role of bacteria and archaea in the oxidation and reduction of both arsenic and iron in these thermal systems.

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