Microbial characterization of white mats in a hydrothermally-influenced, sulfur-rich brine pool

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ABSTRACT

Hydrothermal systems have been implicated in origin of life theories and may host some of Earth's most primitive organisms. Studies of these extreme environments can help to provide a context for the origin of life, to determine the chemical and physical limits to life, and to evaluate the compatibility of life on other planets. In this study, a 16S rDNA gene survey was used to characterize the microbial community of the white mats at Hot Lake, a hydrothermally-influenced, submarine brine pool near Panarea Island (Sicily). Clone libraries of environmental DNA revealed a highly diverse community of bacteria dominated by sequences of *Epsilonproteobacteria*. Surprisingly, members of the *Thiotrichales*, which are commonly observed in marine sulfur mats, were not detected. The archaeal clone library was less diverse; most of the archaeal sequences were related to thermophilic and hyperthermophilic organisms but no Crenarchaeota were found. Overall, the data show that the microbiology is intricately tied to the geochemistry of Hot Lake; it is likely that elemental sulfur filaments in the white mats are biologically precipitated as part of a microbially-mediated sulfur cycle. Future investigations combining quantitative approaches with culturing can help better assess the microbial abundances, community structure, and metabolic activities at Hot Lake.

INTRODUCTION:

Deep-sea hydrothermal systems and the deep subsurface biosphere appear to be some of the most inhospitable environments on Earth and yet they have been shown to host abundant microbial communities (e.g. Corre et al. 2001; Takai et al., 2003; Crépeau et al., 2011). In fact, these high-temperature, high-pressure, hypersaline, extreme-pH environments have been implicated in origin of life theories meaning that the microbes living there may be closely related to the earliest life forms (Wächtershäuser, 2006; Martin et al., 2008). Studies of deep-sea hydrothermal systems and the deep subsurface are therefore useful in providing clues to the context for the evolution of life as well as the chemical and physical limits to life. Unfortunately, the deep-ocean is difficult and expensive to access, but shallow-sea hydrothermal systems exhibit similar geochemical characteristics and can serve as important analogues for the study of their deep-sea counterparts.

At hydrothermal vents, hot reducing fluids from the subsurface mix with seawater to generate chemical disequilibria. Chemolithotrophs, which represent the close link between the biosphere and the lithosphere, take advantage of these disequilibria to obtain energy through the coupling of redox reactions. For example, *Halothiobacillus kellyi*, isolated from the Aegean Sea, is a strictly aerobic bacterium capable of utilizing thiosulfate, tetrathionate, sulfur or sulfide as an electron donor (Sievert et al. 2000a). The rich communities of archaea and bacteria in hydrothermal systems are typically thermophilic or hyperthermophilic in adaptation to the extreme conditions in which they must survive. Many hydrothermal systems are characterized by the presence of white microbial mats, which are of particular interest because they host a consortium of organisms involved in complex interaction in the sulfur- and other biogeochemical cycles (e.g. Taylor et al., 1999; Sievert et al., 1999; Crépeau et al., 2011). Organisms detected in these ubiquitous white mats include *Beggiatoa* and *Thiothrix* which are

thought to biologically precipitate the elemental sulfur that gives the mats their yellow-white color (e.g. Maugeri et al. 2009; Gugliandolo et al. 2006). Nevertheless, the microbial composition and ecological role of the white mats are unique to each hydrothermal vent system with distinct physico-chemical conditions. This investigation focuses on characterizing the microbial community of the white mats at Hot Lake (Lago Caldo), a shallow-sea hydrothermal system off the coast of Panarea Island, Italy.

Since an estimated 99% of microorganisms in the environment cannot be cultivated using standard techniques (Amann et al., 1995), culture independent methods are often used to characterize microbial communities. In this study genetic material isolated from environmental samples is systematically analyzed and classified to produce a genetic library of microorganisms from a shallow-sea hydrothermal system. Here, the genetic sequence encoding the 16S small ribosomal subunit is used as a basis for phylogenetic comparison because it has become widely recognized as an accurate diagnostic tool for classification (Woese and Fox, 1977; Lane et al. 1985). The 16S rDNA sequence contains hypervariable "loop" regions that can provide species-specific signatures as well as highly conserved "stem" regions characteristic of higher-order phylogenetic branches. It is also relatively short (~1500 base pairs) and universally distributed in all prokaryotes which is why 16S gene surveys are commonly used for microbial diversity assessments (e.g. Sievert et al., 2000b; Rogers and Amend, 2005; Hirayama et al. 2007).

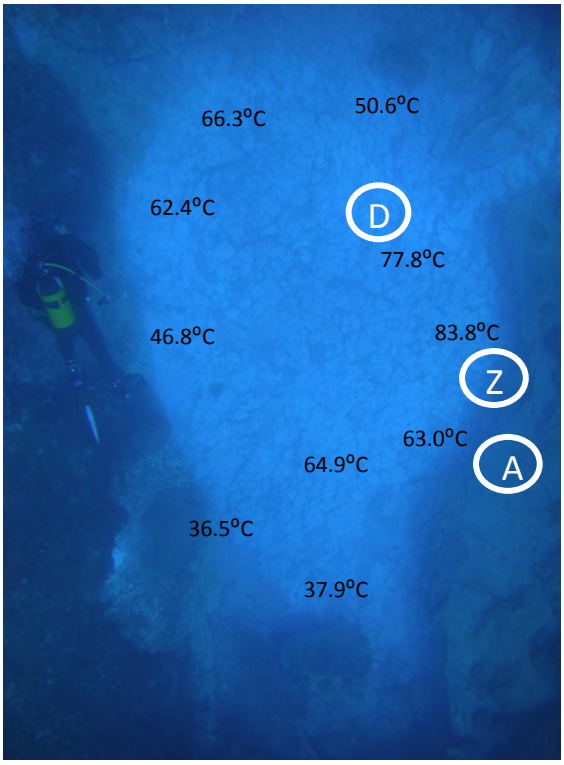


Figure 1: View of Hot Lake from above, with individual sample sites labeled A, D, and Z. Temperature data taken from Leidig and Barth, 2009 were measured at sediment depths of 50 cm with ambient water temperature at 25°C.

BACKGROUND:

Panarea is one of seven volcanic islands in the in Aeolian Archipelago; two of these island are still volcanically active (Stromboli and Vulcano) and hydrothermal activity has been reported throughout the Aeolian Arc (Italiano, 2009). The hydrothermal vents on Vulcano and in the surrounding off-shore region support anaerobic and aerobic, thermophilic and hyperthermophilic archaea and bacteria, most of which have been identified by DNA extraction and have no cultured close relatives (Amend, 2009; Maugeri et al. 2009; Gugliandolo et al. 2006).

Hot Lake is a shallow submarine brine pool located about 2.5 km off the coast of Panarea Island. The circa 10 x 6 m shallow (~2.5 m deep) depression, at a water depth of 20 m,

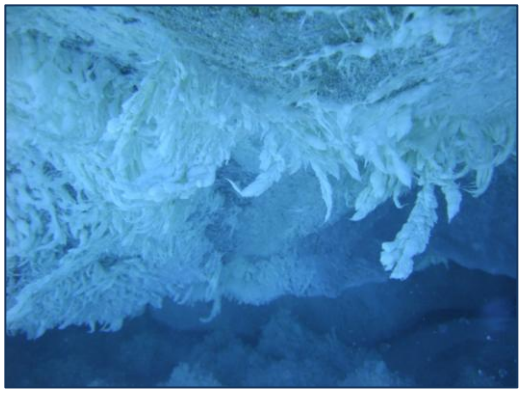


Figure 2: white precipitates attached to a rock at Hot Lake.

formed in 2002 during a submarine, volcanic explosion (Italiano, 2009). It is filled with dense, sulfur-rich brine thought to be leaking from a reservoir beneath the seafloor. Gas emissions are more than 95% CO_2 and temperatures reach up to 83.8°C, but are not uniform across Hot Lake (Figure 1) indicating high spatial variation in hydrothermal fluid flux. Just below the surface, there is a zone of SO_4^{2-} depletion concurrent with a zone of H_2S enrichment, which could be indicative of microbial sulfate reduction (Huang et al., 2010).

A thick microbial mat, consisting predominantly of elemental sulfur (Figure 2), thrives at the hydrothermal fluid-seawater interface at the bottom of the brine pool, where the sediment surface temperature averages 37°C. This is the first study to characterize the microbial composition of the Hot Lake white filaments, although the local abundance and variety of sulfur species suggest the presence of complex sulfur-metabolizing microbial communities within the mat.

MATERIALS AND METHODS

Sample collection

Samples of the microbial mats were collected in 50 mL Falcon tubes via SCUBA diving at three different sites within Hot Lake and stored at 4°C. Sample A was taken from the rim of Hot Lake, D was taken from the bottom of Hot Lake, and Z was scraped from the rocks near the hottest site (see Figure 1).

DNA extraction, amplification, and cloning

Genomic DNA was extracted from each of the three microbial mat samples. The sulfurous material was centrifuged for 2 min at 16,000 g to obtain a pellet of about 0.25 grams dry weight, and then DNA was extracted according to the protocol from the MOBIO PowerSoil® DNA Isolation Kit. In short, this procedure is designed to mechanically lyse cells via bead-beating and collect their genomic DNA.

Polymerase Chain Reaction (PCR) amplifies the 16S ribosomal RNA gene using bacteria and archaea-specific primers; the universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') were used to target bacteria, and primers 21F (5'-TTC CGG TTG TAC CYG CCG GA-3') and 1391R (5'-GAC GGG CGG TGT GTR CA-3') were used for archaea. PCR conditions for bacterial 16S genes were as follows: denaturing step of 5 min at 95°C; 1 min at 95°C, 1 min annealing step at 52°C, and 1 min primer extension step at 72°C for

35 cycles; and a final extension step of 5 min at 72°C. PCR conditions for members of the archaea were as follows: denaturing step of 5 min at 95°C; 1 min at 95°C, 1 min annealing step at 55°C, and 2 min primer extension step at 72°C for 30 cycles; and a final extension step of 15 min at 72°C. PCR products were visualized on a gel using SYBR green, then excised and purified using the protocol from the Promega gel and PCR clean-up kit.

Archaeal and bacterial PCR products were cloned using the QIAGEN® PCR Cloning Kit according to the manufacturer's instructions. In summary, this procedure involves the ligation (or insertion) of PCR products into a DNA plasmid, the transformation (or uptake) of the plasmid by competent E. coli cells, and culturing of the E. coli on plates to form colonies. In theory, each colony growing on the agar plate originates from a single replicating E. coli cell, and therefore contains multiple copies of a single plasmid insert. Colonies were screened for inserts by blue-white screening for expression of the *lacZ*α gene for the β-galactosidase enzyme, and by visualization on a gel. Plasmid DNA was purified using the QIAGEN Miniprep Kit and then sent to MCLab (South San Francisco, CA) for sequencing.

Phylogenetic analysis

The resulting DNA fragments were checked for quality, trimmed, and assembled into contiguous sequences using the program Sequencher 4.0. PCR can generate chimeric sequences by the fusion of phylogenetically distinct parent sequences during the annealing stage, so all of the sequences were checked for chimeras using the program Bellerophon. Each contiguous sequence was assigned to a group, or operational taxonomic unit (OTU), based on a minimum overlap of 100 base pairs with greater than 97% sequence similarity. Basic Local Alignment Search Tool (BLAST) searches were conducted within the GenBank database (http://www.ncbi.nlm.nih.gov/) to determine sequence relatedness to cultured and uncultured organisms. To ensure that a comprehensive sample of microbial diversity was obtained, rarefaction curves (Figure 3) were constructed by graphing the number of additional clones sequenced against the number of new OTUs detected. A full-length, representative sequence was selected from each distinct group and automatically aligned in BioEdit to similar sequences obtained from the 16S rRNA database greengenes. DNA inserted into a plasmid vector for cloning often produces raw sequences that contain some vector sequence at one or both ends of the DNA of interest. Therefore, alignments were manually checked for vector contamination and trimmed. Maximum parsimony phylogenetic trees were constructed using the program PAUP*4.0.

Microscopy

DAPI (4',6-diamidino-2-phenylindole), is a fluorescent stain that binds strongly to A-T rich regions in DNA allowing for visualization of cells under ultraviolet light. A 1:10 dilution with sterile seawater was prepared from a sample tube of white mat after vortexing for 10 sec to resuspend sulfur precipitates. To fix the sample, 250 µl of formalin (37% formaldehyde) was

added per 500 µl of diluted sample. An equal volume of DAPI (10 ppm) was added to the solution and incubated in the dark for 15 min. Samples were vacuum filtered onto 0.22 µm black GE Polycarbonate filters and visualized under UV light at 1,000X magnification.

RESULTS AND DISCUSSION:

A total of 277 bacterial clones and 196 archaeal clones were sequenced; of these, 93, 92, and 92 bacterial clones and 74, 73, and 49 archaeal clones were sequenced from sites A, D, and Z, respectively. Figure 3 shows the rarefaction curves used to determine sufficient sequencing from each site. The rarefaction curves for bacterial clones at sites A and D do not reach a horizontal asymptote, showing that even after sequencing 92 clones from each site, I cannot be confident that the complete diversity is represented here. No additional bacterial clones were sequenced from sites A and D after only 2 new OTUs were detected from the last 18 clones sequenced. All other rarefaction curves approach a horizontal asymptote. It can also be seen that diversity is lower for archaea than the bacteria at all sites. The level of bacterial diversity is relatively higher at sites A and D than at the hot site Z; only 22 OTUs were found at Z compared with 27 and 29 at A and D respectively.

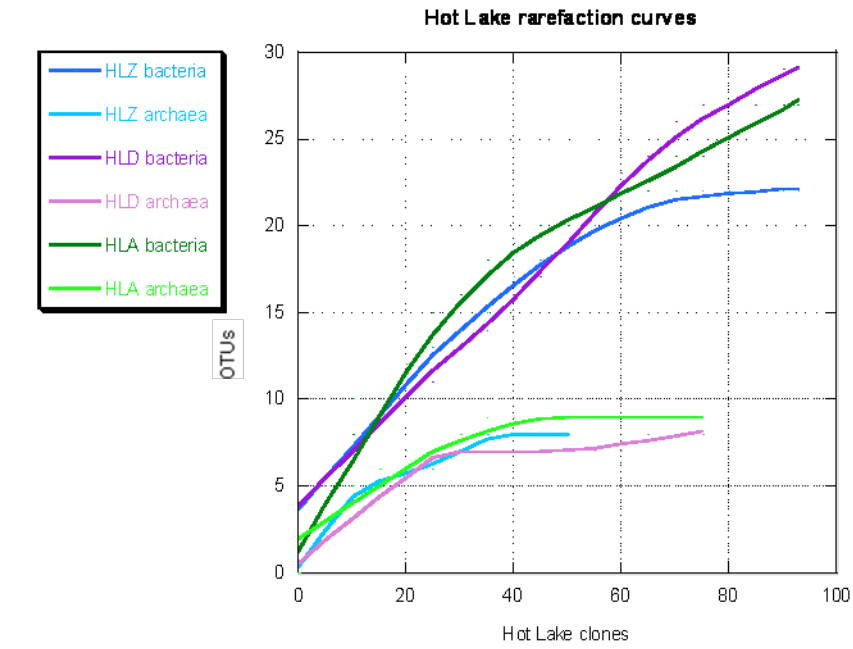


Figure 3: Bacterial and archaeal diversity, represented by the number of OTUs, is shown for each sample site. Curves approaching a horizontal asymptote indicate that for each new clone sequenced, no new OTUs were detected, and therefore the full diversity of the site is represented.

Bacteria

Bacterial sequences were assembled into 261 full contiguous sequences (16 sequence fragments were not included), of which 233 were determined to be non-chimeric. These were assigned to 67 distinct OTUs, of which more than half contained only one clone. A representative sequence selected from each OTU is included in the maximum parsimony phylogenetic tree in Figure 4a, illustrating the bacterial diversity at Hot Lake. An expanded phylogenetic tree for the *Epsilonproteobacteria* is shown in Figure 4b. Most groups are

supported by high bootstrap values. One clone related to *Nitratiruptor* forms a monophyletic group which may be a long branch artefact (Bergsten, 2005). The clade of *Campylobacterales* contains a large number of clones and many taxonomic subdivisions, but there are few similar

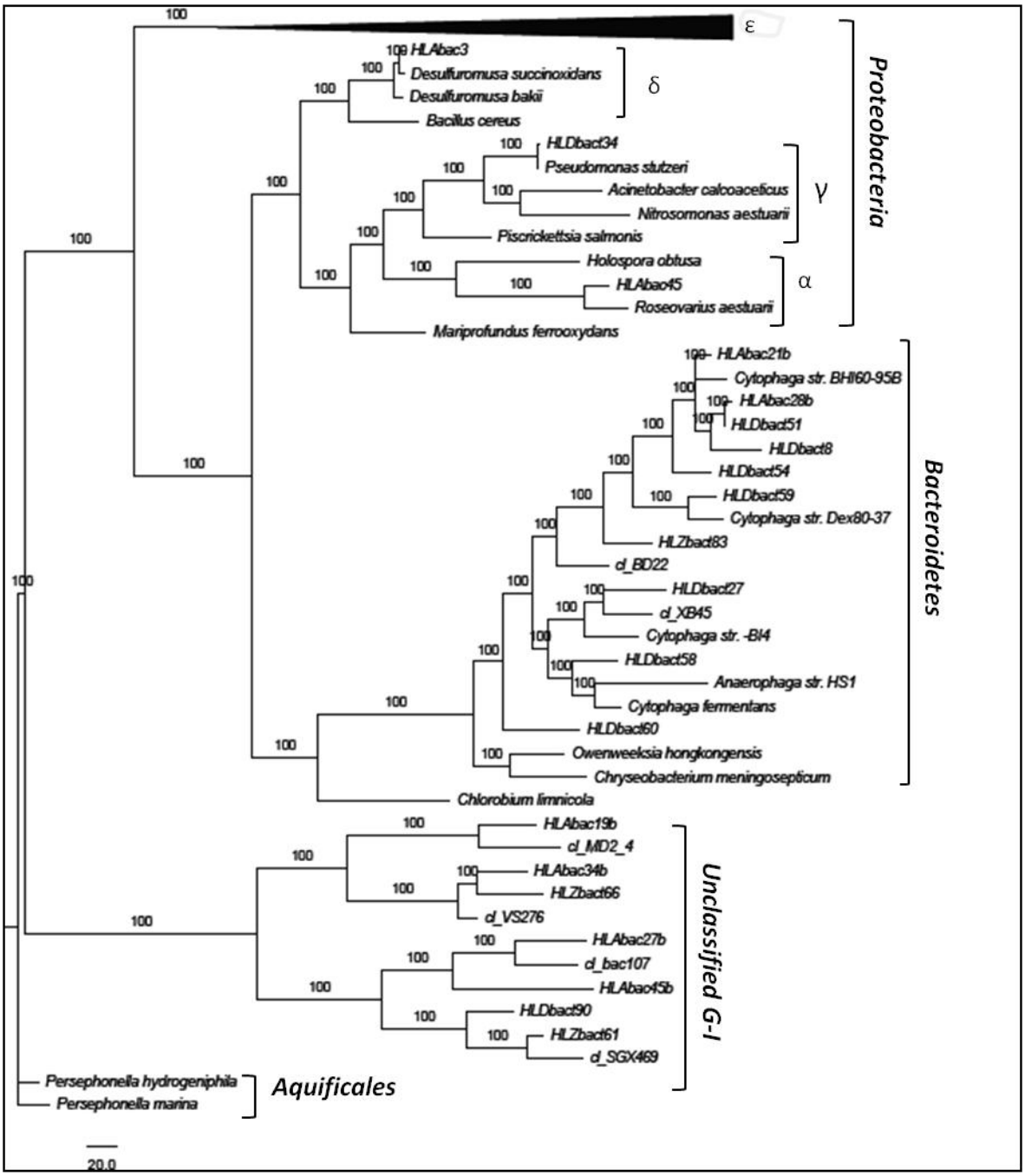


Figure 4a: Maximum parsimony phylogenetic tree from 16S rRNA gene sequences (1000 random addition replicates). Bootstrap values indicate 100 parsimony replicates and scale bar indicates the number of steps. Collapsed clade of"*ε-Proteobacteria*" comprises 47 OTUs from Hot Lake, with *Aquificales* as the outgroup.

isolates to support the topology. A cluster of 7 representative sequences with little similarity to any known, cultured organisms were classified as a separate, deep branch (*Unclassified G-I*) within the bacterial tree. Another group of 10 representative sequences were similar to uncultured clones of the *Cytophagia* within the phylum *Bacteroidetes*. The greatest amount of diversity was observed within the *Epsilonproteobacteria*. The *Epsilonproteobacteria* branched

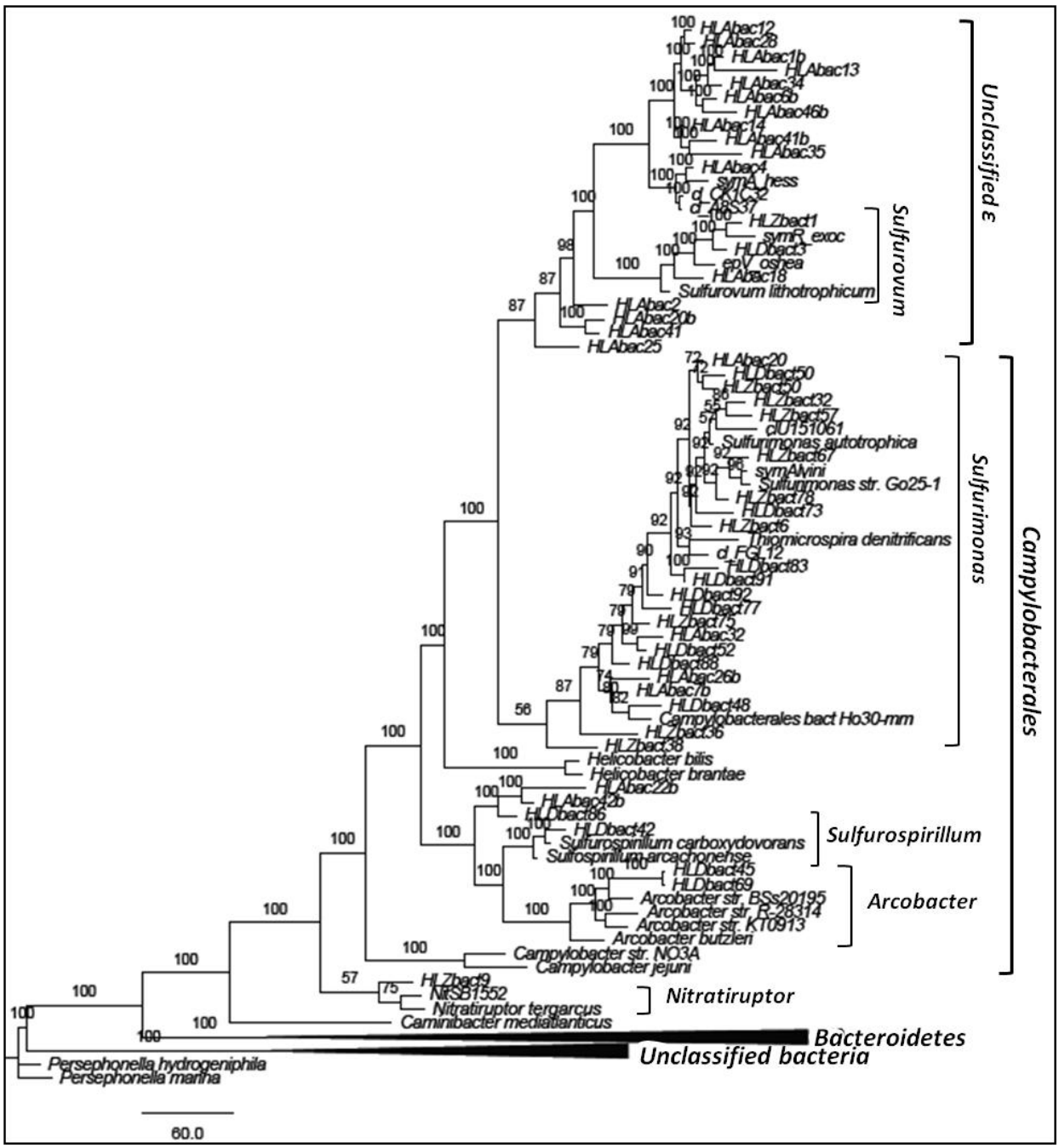


Figure 4b: Maximum parsimony phylogenetic tree of *ε-Proteobacteria* from 16S rRNA gene sequences (1000 random addition replicates). Bootstrap values indicate 100 parsimony replicates and scale bar indicates the number of steps.

separately from the other *Proteobacteria* (α,β,δ and γ) and the *Bacteroidetes*, even when different backbone and outgroup sequences were used (data not shown). In the literature, 16S phylogenetic trees do not exhibit consistent topologies for the five *Proteobacterial* subdivisions (e.g. Corre et al., 2001; Hiorns et al., 1997; Glöckner et al., 2000; Sievert et al., 2000b). This may be due to the fact that few class-specific signature 16S rRNA sequences of the *Proteobacteria* have been determined compared with those found in other major bacterial lineages (Woese, 1987). Topological discrepancies can also arise from the different algorithms used in computer programs for constructing maximum parsimony phylogenetic trees.

To create a better picture of the spatial variation of bacterial diversity in the white mat, the bacterial clone libraries from each site were represented as pie charts (Figure 5). It can be seen that the bacterial community compositions are similar across the three sites. All of the clone libraries were dominated by sequences affiliated with the *Epsilonproteobacteria*, with lesser numbers of *Cytophagia* and uncultured, unclassified bacteria. However, it is important to note that the resolution, or taxonomic level chosen to represent diversity, affects the total apparent diversity. In Figure 5, bacterial sequences are grouped by taxonomic class, but this ignores the huge amount of species richness existing within the *Epsilonproteobacteria*.

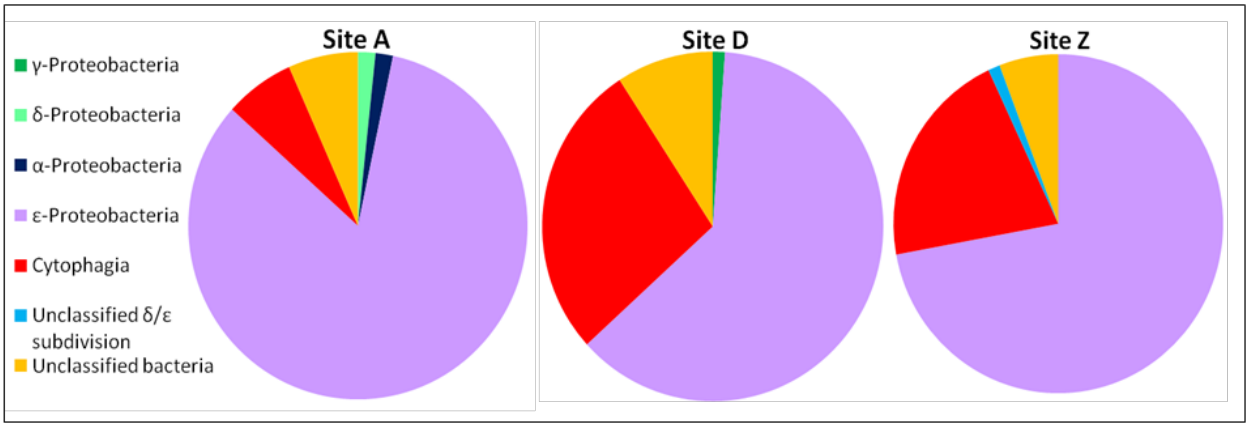
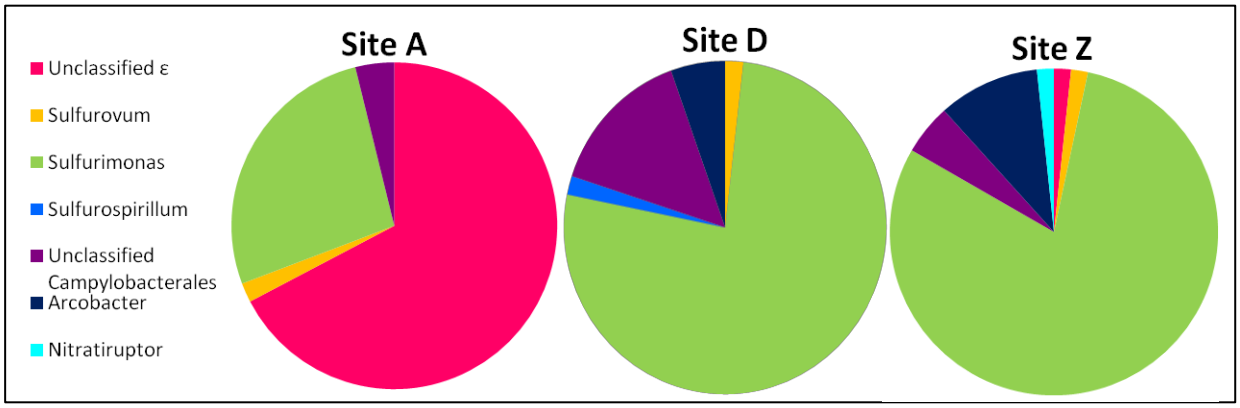


Figure 5: Relative abundances of Hot Lake bacterial sequences from each sample site. Taxa are assigned at the class level based on closest match BLAST results and position on maximum parsimony phylogenetic trees

Alternatively, taxa can be assigned at the genus level as illustrated in Figure 6. It is evident that significant differences exist in the distribution *Epsilonproteobacteria* across the Hot Lake sites. Sequences related to a group of unclassified *Epsilonproteobacteria* were dominant at site A, whereas sequences related to the *Sulfurimonas* were dominant at sites D and Z. While some taxa were represented at all sites, sequences of *Arcobacter* were not found at site A, and sequences of *Sulfurospirillum* and *Nitratiruptor* were only found at site D and Z respectively. Possible explanations for this apparent variation will be discussed later in this paper.

The *Epsilonproteobacteria* are commonly found in marine benthic environments and hydrothermal vents (e.g. Corre et al., 2001; Takai et al., 2003). Most of the sequences were

Figure 6: Relative abundances of *ε-Proteobacterial* sequences from each sample site. Taxa are assigned at the genus level based on closest match BLAST results and position on maximum parsimony phylogenetic trees



affiliated with the *Campylobacteriales*, which are generally mesophilic and micro-aerophilic (Miroshnichenko, 2004). A group of clones were closely related to the genus *Arcobacter* which includes species of human pathogens as well as bacteria isolated from sewage, oil fields and saline environments (Boone et al., 2005). *Arcobacter* are capable of producing elemental sulfur in the filamentous form from the oxidation of sulfide (Wirsen et al., 2002). Many sequences were related to the genus *Sulfurimonas*, comprising sulfide-oxidizing species such as *S. denitrificans* which couples the oxidation of thiosulfate with the reduction of nitrate (Timmer-ten Hoor, 1975). A few clones were affiliated with the genus *Sulfurovum,* also sulfur-oxidizing chemolithoautotrophs among the unclassified *Epsilonproteobacteria* (Inagaki et al., 2004). At site Z, one clone was found belonging to an unclassified group in the delta/epsilon subdivision of the *Proteobacteria* and was 94% similar to the isolate *Nitratiruptor tergarcus*. This organism performs respiratory nitrate reduction with H_2, forming N_2 as a metabolic product and is able to use O_2 as an alternative electron acceptor for growth (Nakagawa et al., 2005).

The predominance of sulfur-metabolizing organisms in the white mat is indicative of a complex, biologically-mediated sulfur-cycle within Hot Lake. In fact, it is likely that sulfide-oxidizers in the microbial mat are responsible for the precipitation of elemental sulfur at the bottom of the brine pool. Characterization of the white mats by scanning electron microscopy (Figure 7) revealed elemental sulfur crystals of various morphologies – spherules of seemingly biotic origin and orthorhombic forms more typical of abiotic crystallization processes. The spherical sulfur particles were very similar to bioprecipitates from the large sulfur-bacteria of the family *Thiotrichaceae* (Larkin, 1983). For example, biomineralized sulfur produced by *Thioploca*, is bonded in the stable S_8 ring configuration and occurs as spherules composed of extremely fine-grained microcrystalline granules (Pasteris et al., 2001). In the same study it was also found that over time (from 1-48 hrs), the sulfur spherules aggregated into clusters, and later developed into individual, polygonal crystals. The sulfur from Hot Lake may undergo similar development under microaerobic conditions; platy crystals could result from the

dissolution and recrystallization of biological precipitates. This is supported by the finding of long filamentous bacteria typical of the *Thiotrix* and *Beggiatoa* morphology in DAPI images of the mat (Figure 8).

Surprisingly, no *Thiotrichales* were found in the mat clone libraries although the filamentous, sulfide-oxidizing bacteria *Thiothrix* and *Beggiatoa* are commonly observed in marine sulfur mats (Teske & Nelson, 2006; Schulz et al., 1999). It could be that the methods used here simply failed to detect the *Gammaproteobacteria* ribosomal DNA. Oftentimes, DNA extraction techniques, specific primers used, or preferential amplification of certain DNA sequences during PCR can influence what genetic information is obtained from environmental samples. A new study by Huang et al. is currently attempting to detect the *Gamma-proteobacteria* using 454 Pyrosequencing. This method has the advantage of greater throughput and sensitivity than conventional sequencing, and is therefore expected to produce a better coverage of the white mat microbial community.

Although the sulfur-oxidizing members of the *Epsilonproteobacteria* are by far the best represented in the clone library, it appears that the Hot Lake environment also supports a wide variety of metabolisms, including chemotrophy, organotrophy, and phototrophy. The second most abundant group of sequences belonged to the *Cytophagia* which are non-fruiting myxobacteria ("myxo"= mucus-producing) ranging from aerobic to facultatively anaerobic (Stanier, 1947; Kaiser et al., 2010). All *Cytophagales* are organotrophs and have

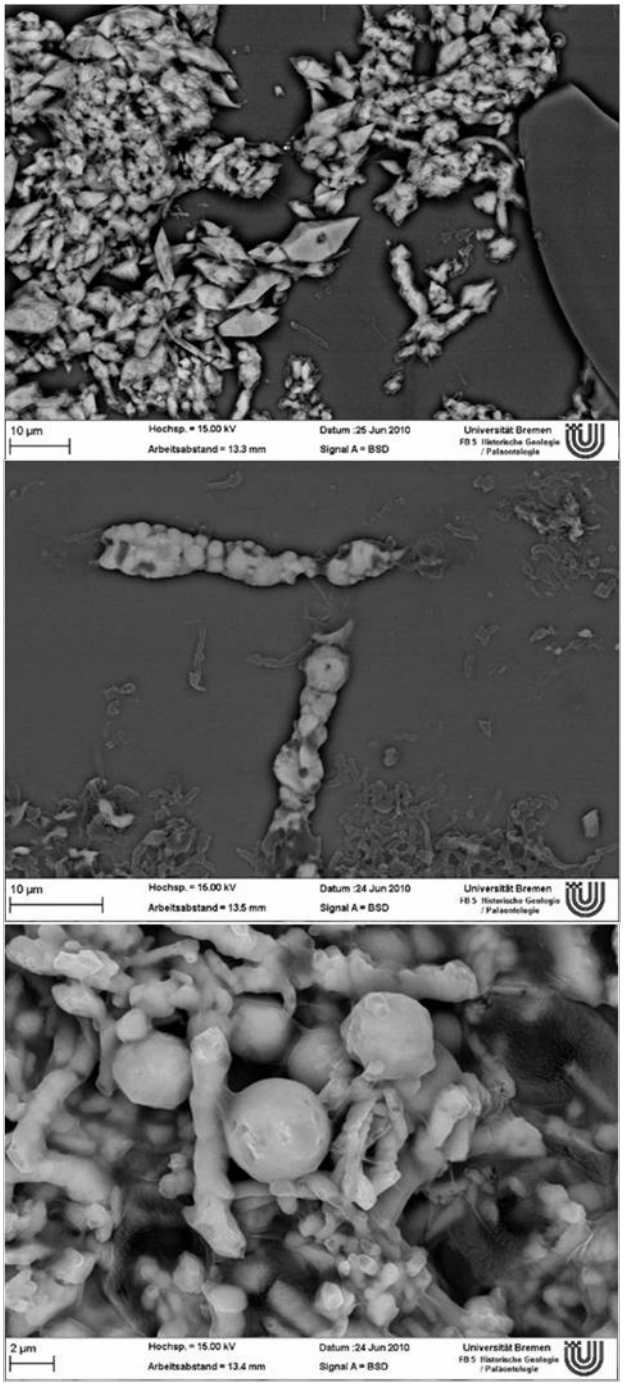


Figure 7: SEM images of white mat showing elemental sulfur particles of various morphologies; (*top*) platy S^0-crystals up to 10µm in length, (*middle*) globular aggregates; (*bottom*) spherules averaging 2-4 µm in diameter (Huang, personal communication).

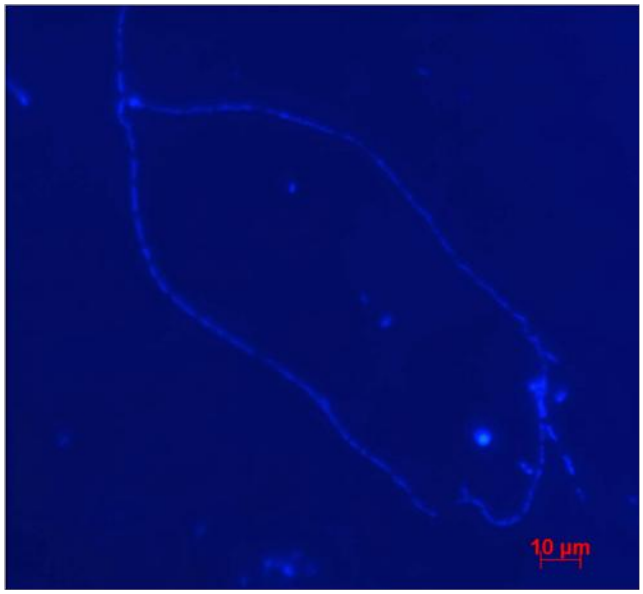


Figure 8: Filamentous cells from white mats visualized by DAPI epifluorescence microscopy under 1000X magnification. Bar is 10 µm.

been found to degrade biomacromolecules such as proteins, chitin, pectin, agar, starch, and cellulose in marine sediments (Reichenbach, 2006). It can be inferred that these organisms are involved in the turnover of organic matter in the Hot Lake system.

Sequences affiliated with the *Alphaproteobacteria* and the *Deltaproteobacteria* were only found at site A. One clone from A was 96% similar to *Roseovarius aestuarii*, a facultatively photoheterotrophic and photoautotrophic *Alphaproteobacterium* in the order *Rhodobacterales* (Biebl et al., 2005). Since the microbial mat is exposed to low levels of sunlight it is not surprising to find evidence of photosynthetic bacteria there. A second clone from A was 98% similar to *Desulfuromusa bakii* in the order *Desulfuromonadales*. This *δ-Proteobacterium* is an obligately anaerobic, sulfur-reducer (Liesack and Finster, 1994). Also, one *γ-Proteobacterium* showing 99% similarity to the cultured organism *Pseudomonas stutzeri* was found at site D. Members of the *Pseudomonadales* tend to be metabolically versatile and include marine bacteria as well as opportunistic plant and human pathogens (Baumann, 1983).

Archaea

Archaeal sequences were assembled into 189 full contiguous sequences (7 sequence fragments were not included), and 181 of these were determined to be non-chimeric. The sequences were assigned to 15 distinct OTUs, the majority of which contained clones from each of the sample

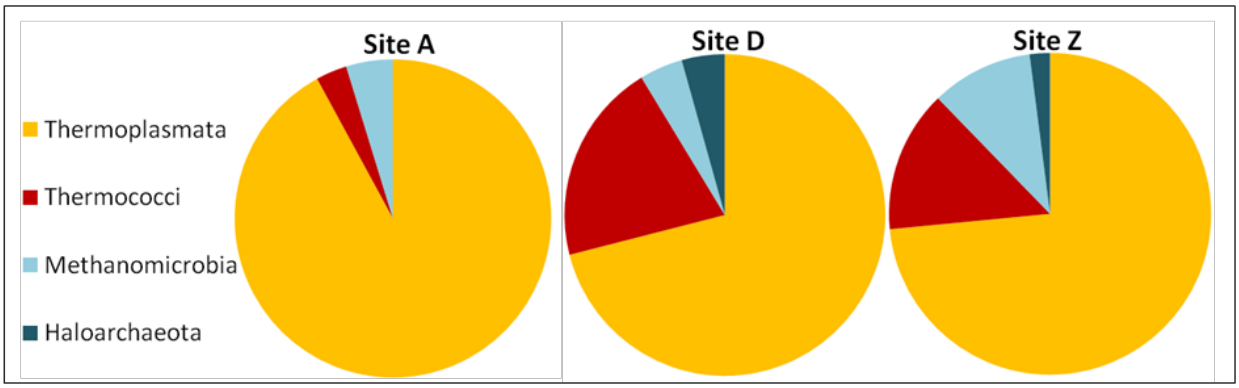


Figure 9: Relative abundances of Hot Lake archaeal sequences from each site. Taxa assigned at the class level based on closest match BLAST results and position on maximum parsimony trees.

sites. The archaeal community did not exhibit any notable spatial variation (Figure 9). Archaeal clone libraries from all of the sites were dominated by sequences related to the *Thermoplasmata*, with minor percentages of *Thermococci*, and *Methanomicrobia*. Sequences affiliated with the *Haloarcheaota* were only found at Hot Lake sites D and Z. No Crenarchaeota were detected.

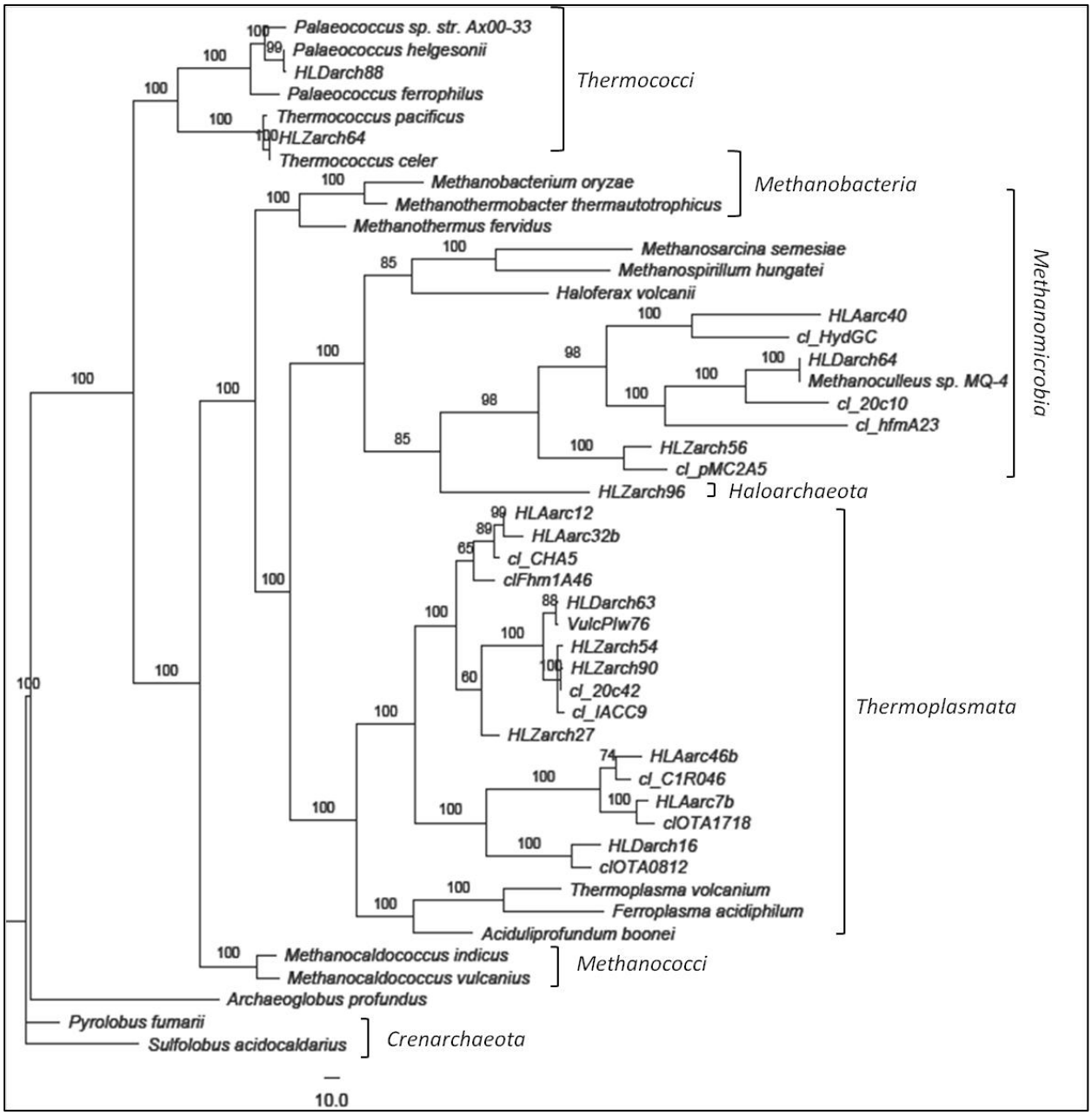


Figure 10: Maximum parsimony phylogenetic tree from 16S rRNA gene sequences (1000 random addition replicates). Two Crenarchaeota, *Pyrolobus fumarii* and *Sulfolobus acidocaldarius* were used as the outgroup. Bootstrap values indicate 100 parsimony replicates and scale bar indicates the number of steps.

The maximum parsimony phylogenetic tree in Figure 10 illustrates the archaeal diversity at Hot Lake. Most groups are well-supported by bootstrap analysis. However, low bootstrap values for two branches of *Thermoplasmatales* probably reflects the fact that none of the Hot Lake clones affiliated with this clade were closely related to any cultured organisms. The greatest archaeal diversity was found among the *Thermoplasmata*, which includes organisms that are acidophilic and thermophilic, and apparently well-suited to the Hot Lake environment. More than 20 sequences were affiliated with the *Thermococci* and clustered into two distinct OTUs; *Thermococcus celer* and *Palaeococcus helgesonii* were the closest relatives to these OTUs by >99% similarity. The *Thermococci* are hyperthermophilic, anaerobic organisms typically found in submarine hydrothermal vents and the deep subsurface (Bertoldo and Antranikian, 2006). In addition, almost 7% of the archaeal sequences were found to belong to the class *Methanomicrobia*, suggesting microbial methane production at Hot Lake. However, the ecological importance of archaea in the system is probably overshadowed by bacterial activity, as explained below.

The Crenarchaeaota are abundant in marine environments especially around hydrothermal vents, and they have been detected in the underlying sediments at Hot Lake, but not in the white mats (Hershberger et al., 1996; Takai et al., 2004; Huang et al., 2010). The absence of Crenarcheaota in the mats is somewhat surprising because they are probably the most abundant and widely-distributed Archaea in the world's oceans (Delong, 2003; Takai et al., 2004). Non-extremophilic Crenarchaeota, especially members of the marine Crenarchaeota Group I, have been identified as the dominant Archaea in moderate-temperature, fluid-seawater mixing environments similar to Hot Lake (Takai et al., 2004).

Comparative analyses

In a study of the subsurface microbial community at Hot Lake by Huang et al. (in progress), sediment cores were taken from the brine pool for assessment by a combination of methods including 16S rDNA gene surveys, CARD-FISH (catalyzed reporter deposition-fluorescence *in situ* hybridization), and IPL (intact polar membrane lipid) analyses. A sediment core from a high-temperature site (74°C at sediment depth of 10 cm) was sliced into sections of 0-1 cm, 5-7 cm, and 14-17 cm for the extraction and sequencing of environmental DNA. The resulting 16S rDNA bacterial clone libraries for each sediment layer are compared with the complete white mat clone library in Figure 11. Members of the *Proteobacteria* (*α*, *δ*, *γ*, and *ε*) and *Bacteroidetes* were found in all of the clone libraries. However, it can be seen that bacterial community composition varies as a function of depth. The white mat was dominated by sequences of the *Epsilonproteobacteria* whereas the *Deltaproteobacteria* were dominant in sediment depths of 0-1 cm, and *Bacteroidetes* were dominant in sediment depths of 5-7 cm and 14-17 cm. Some sequences that were found in the sediments belonged to phylotypes not represented in the white mat: *Betaproteobacteria*, *Fusobacteria, Spirochaetes, Verrucomicrobia, Chlorobium,*

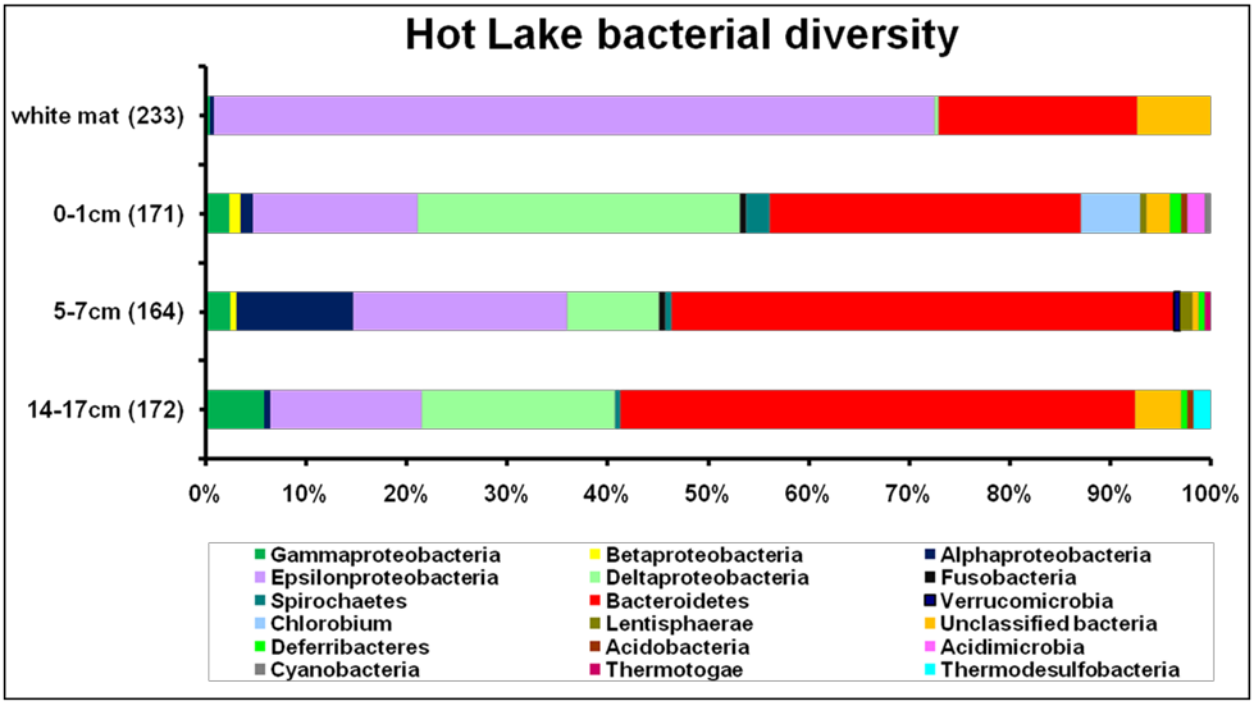


Figure 11: Relative abundances of Hot Lake bacterial sequences, classified based on closest match BLAST results and position on maximum parsimony trees. Values in parentheses indicate number of clones sequenced. Sediment data taken from Huang et al. , in progress.

Lentisphaerae, Deferribacteres, Acidobacteria, *Acidimicrobia, Cyanobacteria, Thermotogae,* and *Thermo-desulfobacteria*.

Figure 12 shows that the Hot Lake archaeal diversity is much less than bacterial diversity, but it also varies with depth. Only two taxa, *Thermoplasmatales* and *Thermococcales*, were represented at all depths. The white mat was dominated by sequences of the *Thermoplasmatales*, whereas the *Halobacteria* were dominant in the sediment depths of 0-1 cm, and the *Thermococcales* were dominant at 5-7 cm and 14-17 cm depth. Sequences belonging to the *Desulfurococcales* were only detected at 14-17 cm depth and no other *Crenarchaeota* were found in the white mats or upper sediment layers.

The variation of diversity with depth likely reflects differences in geochemical conditions due to the hydrothermal fluid-seawater mixing gradient. In contrast, the white mat clone libraries exhibited little variation in diversity at a broad taxonomic level. This could be attributed to the more uniform geochemical conditions existing in the mat; the dilution of hydrothermal fluids by seawater-mixing appears to maintain temperatures at a relatively constant 37°C. However, ocean currents and physical disturbances of the mat are expected to cause at least some degree of geochemical variation. Another factor that may contribute to the relatively uniform diversity across the microbial mat could be the presence of environmentally tolerant bacteria. For example, species in the genus *Sulfurimonas* are able to survive over a broad range of temperatures and oxygen concentrations. *Sulfurimonas denitrificans* has a

rather large genome (2.2 Mbp) that suggests a high degree of metabolic versatility or responsiveness to the environment; in fact, this organism is mixotrophic, capable of growth with a variety of electron donors such as hydrogen, formate, and reduced sulfur compounds (Sievert et al., 2008). Interestingly, significant spatial variation was observed at the genus level of the *Epsilonproteobacterial* clone libraries. Most of the *Epsilonproteobacteria* are sulfur-oxidizers, but each may occupy a specialized ecological niche determined by pH, trace element levels, solar radiation, trophic relationships and competition.

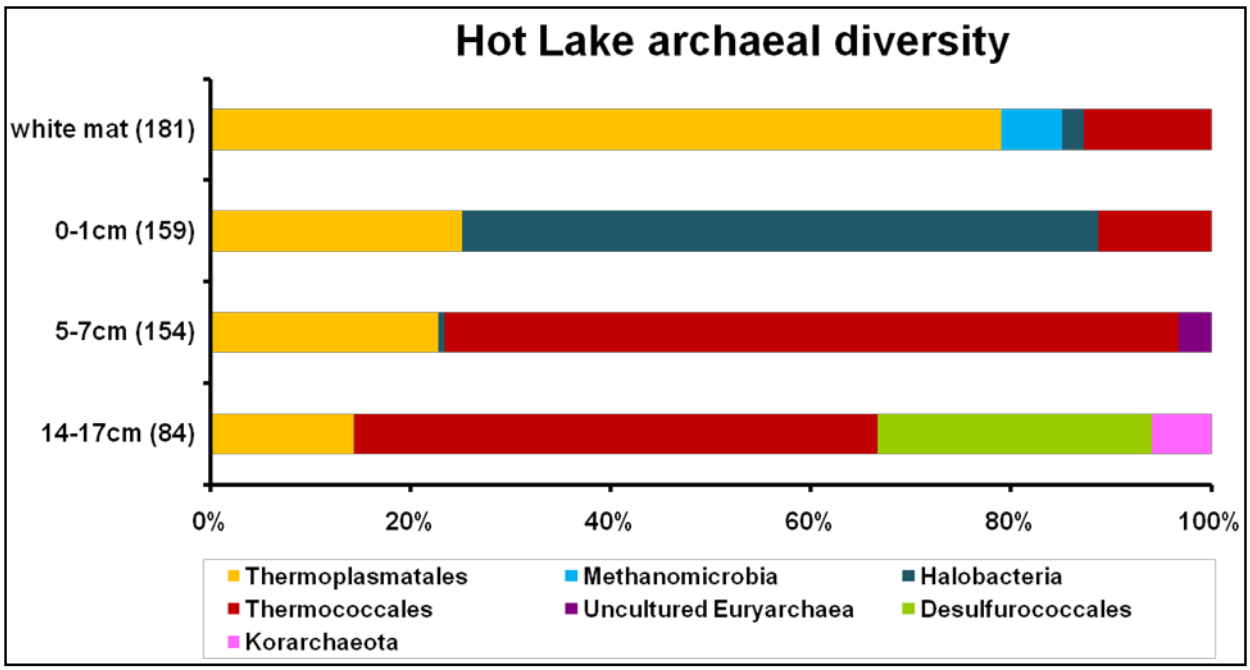


Figure 12: Relative abundances of Hot Lake archaeal sequences, classified based on closest match BLAST results and position on maximum parsimony trees. Values in parentheses indicate number of clones sequenced. Sediment data taken from Huang et al. , in progress.

Diversity is both a function of species richness and evenness. In the discussion of diversity above, these factors were not considered because the 16S sequence data presented here do not necessarily reflect microbial abundances in the natural environment. PCR introduces an amplification bias which renders the data better suited for qualitative assessments. However, analyses of intact polar membrane lipids (IPLs) can be used as a proxy for bacterial and archaeal abundances in the environment. The membrane glycerol esters and ethers with attached polar headgroups produced by prokaryotes are characteristic of their taxonomic affiliation and can be used for identification (Sturt et al., 2004). In the Hot Lake study by Huang et al., (2011) IPL analyses found that bacteria in the sediments were much more abundant than the archaea, but that the relative abundance of archaea increased with depth and temperature. No archaeal membrane lipids have been detected in the white mats (Florence Schubotz, personal communication). One explanation could be that abundant bacterial lipids mask the lipid signal of an extremely small archaeal population, given that some

archaeal 16S rDNA was found in the microbial mat. Overall, this highlights the importance of using a combination of molecular methods to produce an accurate characterization of the microbial community.

CONCLUSIONS:

This 16S survey provides some insight into the diversity of the Hot Lake microbial community, and an overview of metabolic activities as inferred by phylogeny. However, it does not provide accurate information about the relative abundances of each species. Future investigations using quantitative analysis, such as FISH (fluorescent in-situ hybridization) or quantitative PCR, could generate a better idea of the dominant metabolisms in this hydrothermal system. Furthermore, quantitative information about the microbial community structure could be used to target the more prevalent organisms in culturing experiments; these microbes are of interest since they are likely to exert a stronger influence on the ecosystem, especially in the cycling of sulfur.

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