5-9-2008

Microbial Colonization of Deep-Sea Hydrothermal Vent Chimneys

Antoine Pagé
Portland State University

Recommended Citation
Pagé, Antoine, "Microbial Colonization of Deep-Sea Hydrothermal Vent Chimneys" (2008). *Dissertations and Theses.* Paper 5975.
https://doi.org/10.15760/etd.7845

MICROBIAL COLONIZATION OF DEEP-SEA HYDROTHERMAL VENT CHIMNEYS

by

ANTOINE PAGÉ

A dissertation submitted in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY
in
BIOLOGY

Portland State University
2008

DISSERTATION APPROVAL

The abstract and dissertation of Antoine Pagé for the Doctor of Philosophy in Biology were presented May 9, 2008, and accepted by the dissertation committee and the doctoral program.

COMMITTEE APPROVALS:

[redacted]
Anna-Louise Reysenbach, Chair

[redacted]
Michael Bartlett

[redacted]
Sherry Cady

[redacted]
Radu Popa

[redacted]
Kenneth Stedman

[redacted]
William Fish,
Representative of the Office of Graduate Studies

DOCTORAL PROGRAM APPROVAL:

[redacted]
Luis Ruedas, Director
Biology Ph.D. Program

ABSTRACT

An abstract of the dissertation of Antoine Pagé for the Doctor of Philosophy in Biology presented May 9, 2008.

Title: Microbial Colonization of Deep-Sea Hydrothermal Vent Chimneys

Deep-sea hydrothermal vent chimneys contain some of the most extreme environments supporting life on Earth. In the absence of sunlight, the microbial communities that colonize these deposits are based on energy harvested by chemolithoautotrophs, which exploit the chemical disequilibria created as hydrothermal fluids mix with seawater. Although controversial, several models consider deep-sea vent chimneys as likely habitats for primitive ecosystems that may have developed during the Archean.

The goal of this study was to describe spatial and temporal patterns of microbial colonization in chimneys emitting high temperature (>300°C) fluids. Microbial community composition and structure were evaluated through analyses of 16S rRNA gene sequences recovered from selected samples. This information was compared to parameters reflecting in situ conditions (temperature, mineralogy, chemistry) in order to highlight the potential links between different environmental/biological factors and microbial communities.

A relationship was demonstrated between the depth of microbial colonization and characteristics of thermal gradients within the walls of three chimneys collected at Guaymas Basin. In addition, differences in microbial diversity associated with 4- and 72-day-old deposits suggest that internal cycling of locally synthesized organic carbon contributes to promote microbial succession. Processes controlling this succession were further demonstrated by the identification of a sharp transition in the composition and richness of communities associated with newly formed and mature chimneys of the Juan de Fuca Ridge. Finally, patterns of community composition observed in chimneys of the Eastern Lau Spreading Center and Valu Fa Ridge suggest that differences in hydrothermal fluid chemistry and fluid mixing style have direct effects on microhabitat availability within mature deposits.

Combined assessments of 16S rRNA gene diversity and environmental conditions provided new insights into dynamics of microbial colonization in high temperature chimneys. In particular, the deployment of instruments allowing the measurement of in situ conditions within the walls of actively forming deposits constitutes a promising approach to define the parameters that constrain microbial life in these environments. Replicating these studies in various settings will provide a higher resolution understanding of the links between natural phenomena ranging from large-scale geological processes to microscopic interactions at deep-sea hydrothermal vents.

DEDICATION

I want to dedicate this dissertation to my parents and my sister. I moved to Portland by myself, but I was never alone.

ACKNOWLEDGEMENTS

I wish to thank my committee members for their generosity with their time. Special thanks to my advisor Dr. Anna-Louise Reysenbach for having helped me become a better scientist. Thanks you Dr. Michael Bartlett, Dr. Sherry Cady, Dr. William Fish, Dr., Radu Popa, and Dr. Kenneth Stedman for agreeing to serve on my committee.

I would also like to acknowledge collaborators who have contributed to make my work what it is. Dr. Margaret Tivey, Dr. Debra Stakes, Dr. William Seyfried, Dr. Jeffrey Seewald, Dr. Geoffrey Wheat, and Dr. Michael Mottl provided insights that were crucial in the development of the studies I conducted.

Special thanks to my family and friends who have supported me and who have shared the ups and downs of my life during my stay in Portland.

Finally, I would like to thank funding agencies (NSF, FQRNT bourse doctorale) that have supported my work through grants and scholarships awarded to my advisor and me.

TABLE OF CONTENTS

Acknowledgements ii
List of Tables v
List of Figures vi

CHAPTER 1: Introduction
1. Microbial Ecology of High Temperature Deep-Sea Hydrothermal Vent Chimneys 1
1.1. Microbial Communities and their *In Situ* Activity 3
1.1.1. Culture-dependent Analyses 3
1.1.1.1. Archaea 4
1.1.1.2. Bacteria 5
1.1.2. Culture-independent Analyses 6
1.1.2.1. Small Subunit Ribosomal RNA Genes 6
1.1.2.2. Functional Genes 7
1.1.2.3. Stable Isotope Analyses 10
1.1.2.4. Lipid Analyses 10
1.1.2.5. Fluorescent In Situ Hybridization 11
1.2. Factors Influencing Microbial Community Structure in High Temperature Chimneys 12
1.2.1. Physical and Chemical Gradients 12
1.2.1.1. Modeling of In Situ Conditions 12
1.2.1.2. Impact of Physical and Chemical Gradients on Microbial Communities 16
1.2.2. Subseafloor and Seawater Input 20
1.2.3. Biological Interactions 22
1.3. Context of Work 23
1.3.1. Historical Perspective on Use of 16S rRNA Genes to Describe Microbial Communities 23
1.3.2. Limitations of the 16S rRNA Gene Approach 24
1.3.3. Dissertation Hypotheses 26
1.3.3.1. Guaymas Basin Project 26
1.3.3.2. Juan de Fuca Ridge Project 28
1.3.3.3. Lau Basin Project 29

CHAPTER 2: Temporal and Spatial Archaeal Colonization of Hydrothermal Vent Deposits 30
2.1. Abstract 30
2.2. Introduction 31
2.3. Experimental Procedures 35
2.4. Results and Discussion 40
2.5. Conclusion 57

CHAPTER 3: Relationships between Microbial and Mineralogical Diversity in Deep-Sea Hydrothermal Vent Deposits 58
3.1. Abstract 58
3.2. Introduction 59
3.3. Materials and Methods 62
3.4. Results 67
3.5. Discussion 72
3.6. Conclusion 79

CHAPTER 4: Microbial Communities Associated with High Temperature Chimneys of the Eastern Lau Spreading Center and Valu Fa Ridge 81
4.1. Abstract 81
4.2. Introduction 82
4.3. Materials and Methods 85
4.4. Results 86
4.5. Discussion 96
4.6. Conclusion 101

CHAPTER 5: Conclusion 103

REFERENCES 110

APPENDIX A: Phylogenetic Analyses of Microbial 16S rRNA Genes Associated with Hydrothermal Deposits of Guaymas Basin 143

APPENDIX B: Microbial Diversity Associated with Biofilms Exposed to Hydrothermal Fluids at the Galapagos Rift Vent Field 149

APPENDIX C: Microbial Diversity Associated with Hydrothermal Chimneys of the East Pacific Rise (9°N) Vent Field 156

LIST OF TABLES

Table I. Microbial lineages identified at deep-sea vents by surveys of 16S rRNA genes but containing no cultured representatives from these ecosystems. 8

Table II. Quantifications of 16S rRNA genes in DNA extracts obtained from three chimneys of the Juan de Fuca Ridge. 71

Table III. Characteristics of end-member fluids associated with chimneys collected at the Eastern Lau Spreading Center and Valu Fa Ridge. 90

Table IV. Quantification of 16S rRNA genes in DNA extracts obtained from four chimneys of the Eastern Lau Spreading Center and Valu Fa Ridge 90

Table V. Presence of Archaea and Bacteria in samples collected at the Galapagos Rift. 151

Table VI. Summary of the phylotypes detected in all samples of the Galapagos Rift 152

Table VII. Presence of Archaea and Bacteria in samples collected at 9°N on the East Pacific Rise 158

Table VIII. Archaeal phylotypes obtained in a DGGE analysis of 16S rRNA genes PCR-amplified from genomic DNA extracted of various chimney samples (East Pacific Rise) 158

Table IX. Bacterial phylotypes obtained in a DGGE analysis of 16S rRNA genes PCR-amplified from genomic DNA extracted of various chimney samples (East Pacific Rise) 159

LIST OF FIGURES

Figure 1. Some relationships between parameters potentially influencing the composition and distribution of microbial communities within active chimneys 13

Figure 2. Schematic cross-section of a hydrothermal vent chimney wall, showing potential habitats for chemosynthetic microorganisms 14

Figure 3. Temperature recorded within the deposits during the a) 4-day array deployment at Broken Mushroom (BM4), b) the first 19 days of the 72-day array deployment at Broken Mushroom (BM72) 42

Figure 4. Chimney sample (showing the encased thermocouples exposed on the underside of the recovered thermocouple array) collected after the 4-day array deployment at Broken Mushroom (BM4) 43

Figure 5. Chimney sample (a 2 to 3 cm thick wall separating hot vent fluid from ambient seawater) collected after the 72-day array deployment at Broken Mushroom 45

Figure 6. Chimney sample (showing the encased thermocouples exposed on the underside of the recovered thermocouple array) collected after the 72-day array deployment at Toadstool (TS72) 47

Figure 7. Phylogenetic relationship of archaeal 16S rRNA genes as determined by maximum-likelihood analysis 49

Figure 8. Schematic of sample locations and phylotypes from the three different deep-sea hydrothermal vent deposits from Guaymas Basin, Mexico 50

Figure 9. a) Relative crystallinity of chimney samples collected on the Juan de Fuca Ridge (as indicated by peak intensity measured during XRD analyses). b) Mineral composition and DNA content of the same chimneys. Minerals are identified by their patterns of X-ray diffraction, on the basis of JCPDS 69

Figure 10. a) Phylogenetic relationship of archaeal 16S rRNA genes as determined by a neighbor-joining analysis. b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries 73

Figure 11. a) Phylogenetic relationship of bacterial 16S rRNA genes as determined by a neighbor-joining analysis. b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries 74

Figure 12. Chimney samples collected along the Eastern Lau Spreading Center and Valu Fa Ridge 87

Figure 13. Mineral composition and DNA content of chimney samples collected on the Eastern Lau Spreading Center and Valu Fa Ridge 89

Figure 14. a) Phylogenetic relationship of archaeal 16S rRNA genes as determined by a neighbor-joining analysis. b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries 92

Figure 15. a) Phylogenetic relationship of epsilonproteobacterial 16S rRNA genes as determined by a neighbor-joining analysis. b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries 94

Figure 16. a) Phylogenetic relationship of bacterial 16S rRNA genes as determined by a neighbor-joining analysis. b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries 95

Figure 17. Phylogenetic relationship of archaeal 16S rRNA genes as determined by maximum-likelihood analysis 145

Figure 18. Phylogenetic relationship of epsilonproteobactial 16S rRNA genes as determined by maximum-likelihood analysis 146

Figure 19. Phylogenetic relationship of proteobacterial 16S rRNA genes as determined by maximum-likelihood analysis 147

Figure 20. Phylogenetic relationship of bacterial 16S rRNA genes as determined by maximum-likelihood analysis 148

Figure 21. DGGE profiles of the Galapagos Rift samples (bacterial diversity) 154

Figure 22. DGGE profiles of the Galapagos Rift samples (archaeal diversity) 155

Figure 23. Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the 16S rRNA genes (archaeal and bacterial) amplified from subsamples of the chimney fragment collected at the East Pacific Rise 9°N vent field 160

CHAPTER 1: Introduction

1. Microbial Ecology of High Temperature Deep-Sea Hydrothermal Vent Chimneys

Deep-sea hydrothermal vents are created in various geological settings (e.g. mid-ocean ridges, hot spots, back-arc spreading axes, volcanic arcs) by a thermally driven circulation of fluids through the oceanic crust. As it percolates down towards a network of magma chambers, seawater undergoes a series of equilibration reactions with basalts under extreme temperature and pressure conditions (up to 900°C, several kilobars) (Shock, 1992; Seyfried and Mottl, 1995; Delaney et al., 1998). These water-rock interactions result in the formation of hot and acidic fluids (pH 2 to 5.9) containing high concentrations of dissolved metals and gases (up to mM of Fe, Mn, H_2, H_2S, CO_2, CH_4 per kg of fluid) (Von Damm, 1995). Buoyant hydrothermal fluids are subsequently channeled up to the seafloor along faults and fissures created by volcanic and tectonic activity (Fornari and Embley, 1995). Upon mixing of undiluted fluids (up to ~400°C) with cold and alkaline bottom ocean water, a variety of minerals precipitate out of solution and accumulate to form hydrothermal deposits (commonly called high temperature chimneys or black smokers) (Hannington et al., 1995).

The relatively high permeability of mineral assemblages constituting active chimneys generally allows the advection of hydrothermal fluid and seawater within the structures. This phenomenon generates extreme physical and chemical gradients across the walls of active chimneys (Tivey et al., 1990; Tivey and

McDuff, 1990; Tivey, 1995; McCollom and Shock, 1997; Tivey et al., 2002; Tivey, 2004). In sections of the walls where mixing occur, continuous supplies of reduced chemical species and slow kinetics of oxidation-reduction reactions maintain pore fluids in constant chemical disequilibria (Janecky and Seyfried, 1984; Bowers et al., 1985; Shock, 1992). Chemolithoautotrophic microorganisms that colonize these zones can therefore exploit various redox couples to gain energy and fix inorganic carbon (McCollom and Shock, 1997; Tivey, 2004). This primary production fuels extensive microbial communities comprising mesophilic, thermophilic, and hyperthermophilic Bacteria and Archaea (see reviews by Karl, 1995; Jannasch, 1995; Reysenbach et al., 2002; Miroshnichenko, 2004; Miroshnichenko and Bonch-Osmolovskaya, 2006, Takai et al., 2006).

Ancient deep-sea hydrothermal vents constitute a possible habitat for early ecosystems on Earth. Along with other lines of evidence, this hypothesis is supported by high UV radiation and intense meteorite bombardments that could have prevented life from developing in shallow waters during the Archean (Holm, 1992; Baross, 1998; Schlesinger, 1997); by conditions favorable for prebiotic chemistry (Wächtershäuser, 1988; 1990; Shock et al., 1998; Huber and Wächtershäuser, 1998; Huber et al., 2003) and reductive metabolic reactions (Shock et al., 1998; Amend and Shock, 1998) in the abundant submarine hydrothermal systems of this period (Holm, 1992; Baross, 1996; Schlesinger, 1997); and by the apparently primitive origin of many microorganisms found at deep-sea vents (Woese et al., 1990; Woese, 1998; Pace, 1997). Although changes

in the ocean crust and seawater composition have occurred through time (Schlesinger, 1997; Habicht et al., 2002; Poulton et al., 2004), the study of modern deep-sea hydrothermal vents provides an opportunity to further describe the potential contribution of these environments in the development of life on Earth. The investigation of potential extra-terrestrial habitats for life (Jakosky and Shock, 1998; Chyba and Pillips, 2002; Varnes et al., 2003) also benefits from additional information regarding the links between hydrothermal venting and the development of microbial communities.

1.1. Microbial Communities and their *In Situ* Activity

1.1.1. Culture-dependent Analyses

The isolation and characterization of Archaea and Bacteria provides a proxy to extrapolate on the activity of microbial communities in deep-sea hydrothermal vent chimneys. Based on the assumption that most microbial niches in these structures are occupied by thermophiles (optimal temperature for growth $\geq$45°C), culturing efforts have traditionally focused on high temperature conditions. Although this choice might have delayed the isolation of mesophilic microbial populations (optimal temperature for growth <45°C) with significant roles in the cycling of elements within actively venting sulfide deposits (e.g. *Epsilonproteobacteria*), culturing studies have provided a wealth of information, and to this day constitute the basis of our understanding of microbial physiological diversity at deep-sea vents.

1.1.1.1. Archaea

The characterization of the first Archaea isolated from deep-sea hydrothermal vents closely followed the discovery of these ecosystems in 1977 (Lonsdale, 1977; Ballard, 1977). In 1983, Jones et al. presented a new 'methane-producing bacterium' that had a doubling time of 26 minutes at 85°C. The name *Methanococcus jannaschii* was proposed for this new isolate from the East Pacific Rise (21°N), but it was later amended to *Methanocaldococcus jannaschii* to distinguish members of this thermophilic genus from the mesophilic *Methanococcus* species (Whitman, 2001). In the following years, a series of Archaea were isolated from various vent sites: heterotrophs (e.g. *Staphylothermus marinus*, *Pyrodictium abyssi*, *Desulfurococcus* spp., *Archaeoglobus profundus*, *Thermococcus* spp. *Pyrococcus* spp.), methanogens (e.g. *Methanopyrus kandleri*, *Methanocalcodoccus* spp., *Methanothermus fervidus*), and chemolithoautotrophs (e.g. *Ferroglobus placidus*, *Pyrolobus fumarii*) (see reviews by Jannasch, 1995; Karl, 1995; Baross and Deming, 1995; Stetter, 1996; Reysenbach et al., 2002; Miroshnichenko, 2004; Miroshnichenko and Bonch-Osmolovskaya, 2006). The most interesting developments recently provided by culturing experiments were the isolation of the first hyperthermophile capable of anaerobic acetate-oxidation (*Geoglobus ahangari*, Kashefi et al., 2002), the first obligatory aerobic hyperthermophile (*Aeropyrum camini*, Nakagawa et al., 2004b), and the first obligate thermoacidophile (Reysenbach et al., 2006). These discoveries significantly expand the range of known microhabitats for Archaea at deep-sea

vents. In addition, the culturing of previously undetected *Nanoarchaeum equitans* (Huber at al., 2002) suggests that important lineages might have escaped the culture-independent assessments of microbial diversity.

1.1.1.2. Bacteria

Many of the first observers linked the extraordinarily high biomass found at deep-sea hydrothermal vents to a primary production based on bacterial oxidation of hydrogen sulfide (Corliss et al., 1979; Jannasch and Wirsen, 1979). Consequently, the first hydrothermal vent Bacteria isolated in the 1980s were aerobic sulfide-, sulfur-, and thiosulfate-oxidizers (genera *Thiobacillus*, *Thiomicrospira*, *Beggiatoa*, Ruby et al., 1981; Ruby and Jannasch, 1982; Jannasch and Wirsen, 1985, Nelson et al., 1989). Although the attention of microbiologists was focused on sulfur metabolism during this period, a few other Bacteria were isolated, including methane-oxidizers (*Methylococcus* spp.) and hydrogen-oxidizers (*Hydrogenomonas* spp.) (Jannasch, 1995; Karl, 1995). However, it was not until the late 1990s that microbiologists started to uncover the large diversity of phenotypes present in deep-sea vent bacterial communities. The characterization of the thermophilic fermenter *Thermosipho melaniensis* (Antoine et al., 1997), and sulfur-reducer *Desulfurobacterium thermoautotrophicum* (L'Haridon et al., 1998) initiated a series of studies presenting new heterotrophs (e.g. orders *Thermotogales*, *Clostridiales*, genus *Caldithrix*), sulfur-oxidizing/nitrate-reducing microaerophiles/anaerobes (e.g. order *Aquificales*, class *Epsilonproteobacteria*),

sulfate-reducers (e.g. order *Thermodesulfobacteriales*), iron-reducers (e.g. order *Deferribacteriales*, family *Geobacteraceae*), and aerobic organotrophs (e.g. family *Thermaceae*) (see reviews by Reysenbach et al., 2002; Miroshnichenko, 2004; Miroshnichenko and Bonch-Osmolovskaya, 2006). Among recent studies, the characterization of new *Epsilonproteobacteria* species (Alain et al., 2002b; Miroshnichenko et al., 2002; 2004; Inagaki et al., 2003; 2004; Kodama and Watanabe, 2004; Takai et al. 2004c; 2005a; 2006a; Nakagawa et al., 2005a; b) is particularly important since surveys of 16S ribosomal RNA (rRNA) genes in deep-sea vent environments suggest that this group overwhelmingly dominates bacterial communities (see section 1.1.2.1.). Prior to this work, the *Espilonproteobacteria* were known as a series of widely diverse subdivisions containing environmental 16S rRNA gene sequences, but for the most part with no cultured representatives (Corre et al., 2001).

1.1.2. Culture-independent Analyses

1.1.2.1. Small Subunit Ribosomal RNA Genes

The first survey of 16S rRNA gene diversity in a marine hydrothermal vent environment was conducted on a biofilm exposed to low temperature fluids ($\leq$37°C) at Loihi Seamount (Moyer et al., 1994; 1995; 1998). Operational Taxonomic Units (OTUs, i.e. genes grouped based on restriction fragment patterns) found in the sample belonged to phylogenetic divisions *Epsilonproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and marine

Archaea Groups I and II. Although limited by the sequencing of only 12 bacterial and 4 archaeal genes, this culture-independent study offered a glimpse into the composition and structure of deep-sea vent microbial communities. Subsequent surveys of 16S rRNA gene diversity have added a series of lineages to the list of microbial divisions found at deep-sea vents. Most of these lineages do not contain representatives cultured from hydrothermal vent environments (Table I).

Despite a high degree of variability in the composition of microbial communities in different deep-sea vent samples, general patterns are starting to emerge from the accumulation of 16S rRNA gene surveys (Takai et al., 2006b). In particular, the frequent detection of orders *Thermococcales*, *Methanococcales*, *Archaeoglobales*, and group DHVE2 (*'Aciduliprofundales'*) suggest that niches occupied by these lineages are widely distributed among deep-sea hydrothermal ecosystems. Similarly, the ubiquitous distribution of *Epsilonproteobacteria* highlights their important role in microbial communities. Nevertheless, culture-independent studies designed to test the effects of differences in specific environmental or biological factors on the composition and structure of microbial communities remain rare.

1.1.2.2. Functional Genes

Technical advances in microbial ecology have led to the identification of a series of metabolic genes that can be used to identify the potential for specific microbial processes in the environment. At vents, the sequencing and phylogenetic analysis

Table I. Microbial lineages identified at deep-sea vents by surveys of 16S rRNA genes but containing no cultured representatives from these ecosystems (modified from Takai et al., 2006b).

Division	Location	Reference of first report
Archaea		
DHVA1	Izu-Ogasawara Arc	Takai and Horikoshi, 1999
DHVE1, 3-7	Izu-Ogasawara Arc and Okinawa Trough	Takai and Horikoshi, 1999
DHVE8	East Pacific Rise 13°N	Nercessian et al., 2003
DHVE9	Juan de Fuca Ridge	Pagé et al., 2004
Halobacteriales	Manus Basin	Takai et al., 2001
HWCG	Okinawa Trough	Takai and Horikoshi, 1999
Marine Benthic Group B	Izu-Ogasawara Arc	Takai and Horikoshi, 1999
Marine Benthic Group E	Central Indian Ridge	Takai et al., 2004a
Marine *Crenarchaeota* Group I	Loihi Seamount	Moyer et al., 1995
Marine *Euryarchaeota* Group II	Loihi Seamount	Moyer et al., 1995
Marine Pelagic Archaea Group I	Guaymas Basin	Teske et al., 2002
Methanobacteriales	Guaymas Basin	Teske et al., 2002
Methanomicrobiales	Guaymas Basin	Teske et al., 2002
Methanosarcinales	Guaymas Basin	Teske et al., 2002
Thermoproteales	Juan de Fuca Ridge	Pagé et al., 2004
Bacteria		
Alphaproteobacteria	East Pacific Rise 9°N	Lopez-Garcia et al., 2003
Betaproteobacteria	Mid-Atlantic Ridge	Reysenbach et al., 2000
Candidate division OP1	Guaymas Basin	Teske et al., 2002
Candidate division OP3	Guaymas Basin	Teske et al., 2002
Candidate division OP5	Guaymas Basin	Teske et al., 2002
Candidate division OP8	Guaymas Basin	Teske et al., 2002
Candidate division OP9	Guaymas Basin	Teske et al., 2002
Candidate division OP11	Guaymas Basin	Teske et al., 2002
Cytophaga-Flexibacter-Bacteroides	Mid-Atlantic Ridge	Reysenbach et al., 2000
Epsilonproteobacteria Group C	Mid-Atlantic Ridge	Corre et al., 2001
Gammaproteobacteria	Loihi Seamount	Moyer et al., 1995
Green non-sulfur Bacteria (*Chloroflexi*)	Guaymas Basin	Teske et al., 2002
Myxobacteria	Loihi Seamount	Moyer et al., 1995
Plantomycetales	Guaymas Basin	Teske et al., 2002
Verucomicrobia	Guaymas Basin	Teske et al., 2002

of functional genes has provided an additional perspective on the roles of several uncultured microbial lineages. For example, Campbell et al. (2003) studied the occurrence of genes encoding for enzymes of the reverse tricarboxylic acid (rTCA) cycle in symbiotic microbial communities associated with the polychaete *Alvinella pompejana*. The authors identified two fosmid inserts containing ATP citrate lyase and 16S rRNA genes which belonged to *Epsilonproteobacteria* that have been shown to dominate the symbiotic community (by fluorescence in situ hybridization, Cary et al., 1997). Furthermore, they demonstrated that another component of the rTCA cycle, the gene encoding for 2-oxoglutarate oxidoreductase, was common among microbial communities colonizing *A. pompejana*'s tubes. This study suggests that dominant symbiotic and free-living microorganisms associated with *A. pompejana* are likely chemolithoautotrophs. Similar results were later reported for species of other divisions in the class *Epsilonproteobacteria* (Takai et al., 2005b). Analyses of functional gene (e.g. *dsr*, *mcrA*, *pmoA*) diversity have also revealed the existence of previously unrecognized lineages of sulfate-reducers (Dhillon et al., 2003; Nakagawa et al., 2004a; Nercessian et al., 2005), methanogens, and methanotrophs (Nercessian et al., 2005). In some cases, these analyses have even expanded the list of microbial niches known to be available at deep-sea vents. This is the case of studies by Mehta et al. (2003; 2005), who demonstrated that some clades of the nitrogenase gene *nifH* are endemic to these ecosystems. In a subsequent study, Mehta and

Baross (2006) isolated a deep-sea vent species of the genus *Methanocaldococcus* that can fix N_2 to NH_3 at temperatures up to 92°C.

1.1.2.3. Stable Isotope Analyses

The $\delta^{13}C$ and $\delta^{15}N$ analyses conducted by Campbell et al. (2003) represent one of the few cases where measurements of stable isotope ratios have been used to analyze microbial function at deep-sea vents. In this study, the authors supported their observation of abundant rTCA cycle genes in *A. pompejana*'s episymbiotic microbial communities with ^{13}C depletions (-8.9 to -9.3‰) that are in agreement with the fractionation expected by enzymes of this CO_2 fixation pathway (-8.0 to -12.0‰), and ^{15}N depletions generally associated with primary producers (-2.1 to -2.4‰). Likewise, Teske et al. (2002) obtained results suggesting that anaerobic methane oxidation occurs at Guaymas Basin by combining 16S rRNA gene analyses demonstrating the presence of anaerobic methane oxidizers (ANME-1, -2), and isotopic analyses showing important ^{13}C depletions (-58 to -89‰) in archaeal lipids.

1.1.2.4. Lipid Analyses

Hedrick et al. (1992) conducted the first study dedicated to the assessment of spatial distribution in microbial communities colonizing active chimneys. Through a quantification of bacterial polar lipid fatty acids and archaeal ether

lipids extracted from four layers of a flange, they demonstrated that proportions of bacterial cells decrease with depth within these structures (from 100% of the total lipids on the surface to 6% on the bottom), whereas proportions of Archaea follow the inverse trend. The authors hypothesized that a gradual increase in the exposure to hydrothermal fluids might favor archaeal populations. This trend was later confirmed by a study combining analyses of lipids biomarkers and cell counts following fluorescent *in situ* hybridization (FISH) experiments (Schrenk et al., 2003).

1.1.2.5. Fluorescent *In Situ* Hybridization

Several studies using fluorescent *in situ* hybridization (FISH) have been conducted to identity spatial colonization patterns across active sulfide deposits. Experiments targeting wide phylogenetic ranges (total Bacteria and/or Archaea) have demonstrated that cell concentrations and proportions of Bacteria in communities are higher on the outer surfaces of these structures (up to 10^{10} cells/g of mineral, 98% bacterial cells), and decrease rapidly with depth inside their walls ($\leq 10^4$ cells/g of mineral, 24% bacterial cells). On the other hand, proportions of Archaea increase with depth (up to 76% of the cells in deep mineral layers) (Harmsen et al., 1997a; Chevaldonné and Godfroy, 1997; Takai et al., 2001; Schrenk et al., 2003). Studies targeting specific lineages have also confirmed the presence of Bacteria and Archaea of the genera *Thermotoga*, *Thermosipho*, *Methanopyrus*, *Ignicoccus*, and orders *Aquificales*, *Thermococcales*,

Archaeoglobales, *Methanococcales* (Harmsen et al., 1997b; Schrenk et al., 2003; Nercessian et al., 2004).

1.2. Factors Influencing Microbial Community Structure in High Temperature Chimneys

A general picture of microbial communities in active sulfide deposits is starting to emerge from a collection of individual studies. However, these analyses suggest that striking differences occur in the abundance, composition, and distribution of microbial communities between chimneys that otherwise appear similar (see sections 1.1.2.1., 1.1.2.5., 1.2.1.2.). Attempts at measuring the impact of some of the factors potentially causing this variability have been conducted (e.g. differences in gas concentration between fluids, Nakagawa et al., 2005c). However, chemical, physical, geological, and biological studies of high temperature chimneys suggest that numerous factors are involved in shaping microbial communities in these environments (Fig 1.).

1.2.1. Physical and Chemical Gradients

1.2.1.1. Modeling of *In Situ* Conditions

In 1997, McCollom and Shock published thermodynamic models that established the basic geochemical constraints on colonization of sulfide deposits by microbial primary producers (chemolithoautotrophs). The authors calculated physical and chemical conditions resulting from fluid mixes containing different proportions of

hydrothermal fluids and seawater (batch mixing). Microbial habitats available at different temperatures were then interpreted based on results of Gibbs free energy calculations ($\Delta G = \Delta G^0 + RT \ln Q$) for several oxidation-reduction reactions. By transposing the results of these analyses to a theoretical chimney wall, McCollom and Shock (1997) suggested general features that should be observed in microbial communities colonizing sulfide deposits at sediment-starved mid-ocean ridges.

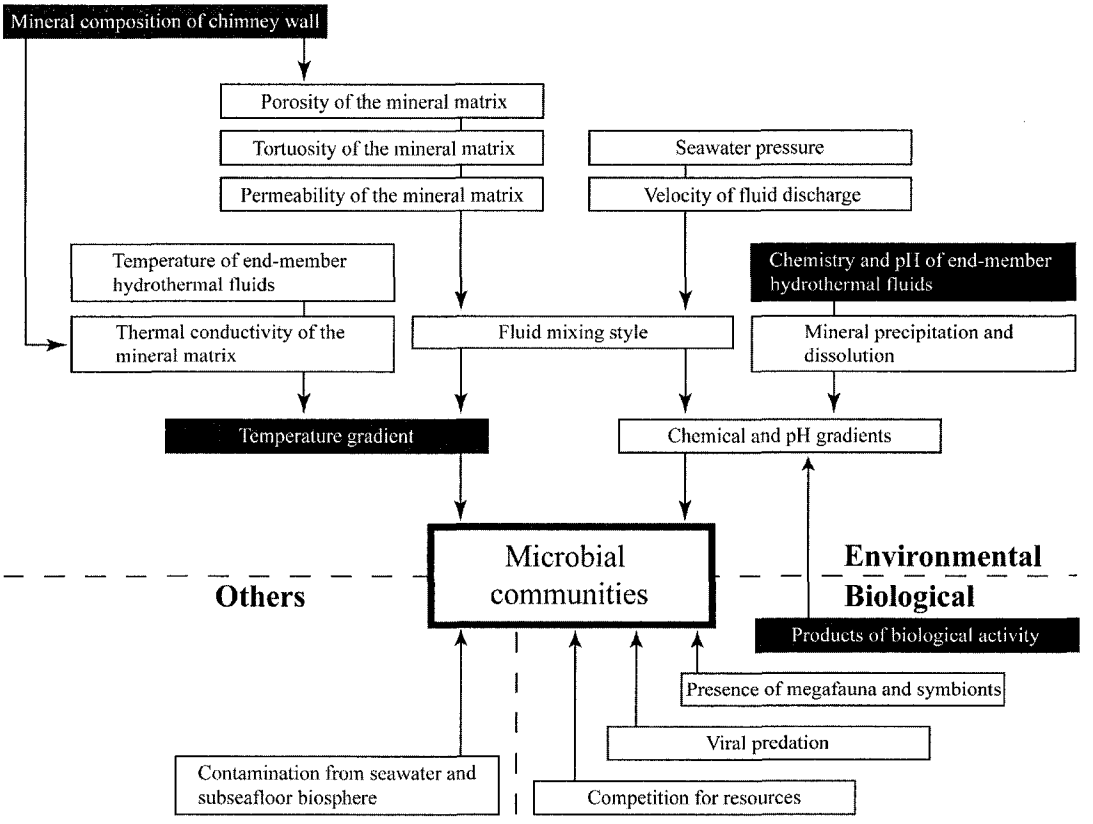

Figure 1. Some relationships between parameters potentially influencing the composition and distribution of microbial communities within active chimneys. In white fonts, factors that were evaluated by the research presented in this document (see section 2.3.).

For example, thermal and chemical gradients resulting from the gradual mixing of hydrothermal fluids and seawater (Tivey et al., 1990; 2002) should stratify microbial habitats and spatially isolate microorganisms according to their physiology. Furthermore, the transition between oxic and anoxic conditions should occur at a temperature of approximately 38°C, which should create habitats only for mesophilic aerobes/microaerophiles, and thermophilic/hyperthermophilic anaerobes. Finally, sulfide oxidation and sulfate reduction should respectively be the dominant aerobic and anaerobic metabolic processes in chemolithoautotrophic populations (Fig. 2).

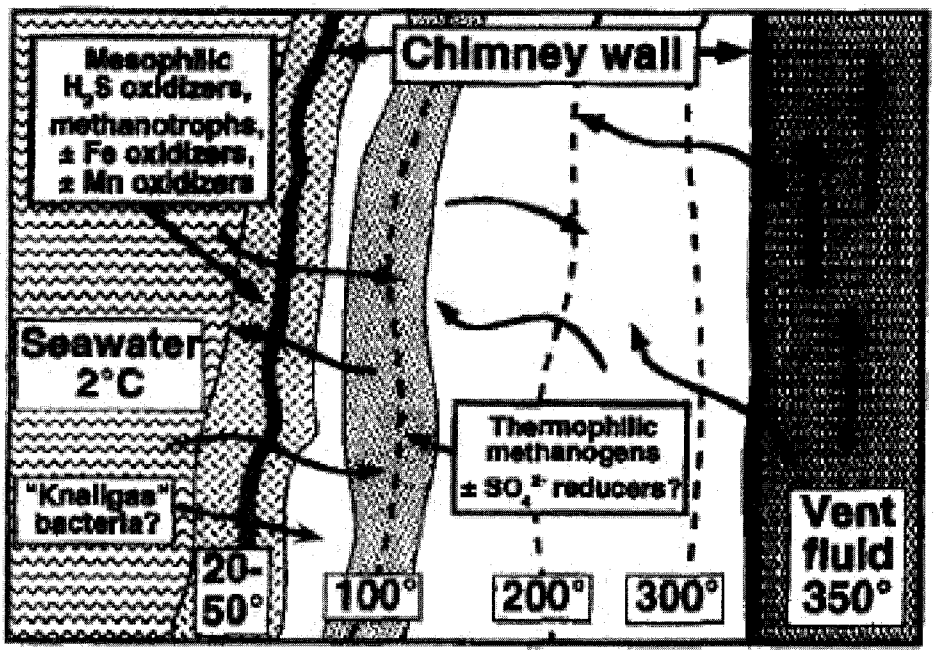

Figure 2. Schematic cross-section of a hydrothermal vent chimney wall, showing potential habitats for chemosynthetic microorganisms (from McCollom and Shock, 1997).

In 2004, Tivey revisited the question of microhabitat availability by conducting additional modeling experiments that considered the diffusive and advective transport of mass and heat across chimney walls. This study took into account multiple physical (thermal conductivity, tortuosity, porosity, and permeability of mineral matrices, fluid velocity, seawater pressure) and chemical parameters (end-member fluid chemistry and pH). Among the most important conclusions provided by the improved models, it was demonstrated that variability in the mineralogical composition of chimney walls, characteristics of end-member (undiluted) fluids, and fluid mixing styles should all have strong effects on the thermal and chemical gradients within black smokers. As a result, environmental conditions and microbial habitats should be far less static than suggested by McCollom and Shock (1997). For example, oxic/anoxic transitions appear to occur under a wide range of temperatures (<3°C to ~90°C), which should allow anaerobic mesophiles and aerobic thermophiles to colonize active structures. This interpretation is supported by the isolation of a strict aerobe growing optimally at a temperature of 85°C (*Aeropyrum camini*, Nakagawa et al., 2004b).

As for thermal and chemical gradients, Tivey (2004) demonstrated that pH gradients are probably affected by both characteristics of end-member fluids and fluid mixing styles. As a result, pH values within the outer sections of active chimneys might vary between ~3 and 8 in deposits emitting classic mid-ocean ridge fluids (end-member pH values ~3-4.5). Tivey's models also predict that pH

values within high temperature environments (~80-120°C) should generally be acidic (<3 to 4.5). Although several hyperthermophiles isolated from active chimneys can grow in such conditions, these microorganisms are generally characterized by near-neutral (6-8) optimal pHs (see review by Reysenbach et al., 2002). This situation led several microbial ecologists to propose that niches for thermoacidophiles might be too narrow to allow the growth of '*Sulfolobus*-type' Archaea in black smokers (Jannasch, 1995). However, the recent discovery of an obligate thermoacidophile belonging to a lineage that appears endemic to deep-sea vents ('*Aciduliprofundum boonei*', Reysenbach et al., 2006) proved these predictions wrong.

1.2.1.2. Impact of Physical and Chemical Gradients on Microbial Communities

The spatial segregation of microbial populations according to their phenotypes is not always observed in sulfide deposits (e.g. Harmsen et al., 1997a). However, major physiological groups generally occupy separate sections of chimney walls (see sections 1.1.2.4. and 1.1.2.5.). Despite the obvious links between these colonization patterns and thermal/chemical gradients, multidisciplinary studies combining assessments of microbial diversity along environmental gradients have not been conducted yet. Thus, the extent to which microbial communities are spatially structured by environmental gradients, the most extreme conditions in which microorganisms can survive within sulfide deposits, and the links between

differences in thermal/chemical gradients and microbial communities in separate chimneys remain unclear.

In 2006, McCliment et al. reported the detection of *Ignicoccus* spp. and *Nanoarchaeum* spp. in a 4-day old chimney with external temperatures ranging from 98°C to 203°C. Although temperatures expected across this chimney are in agreement with the growth conditions of *Ignicoccus* species (70-98°C, Huber et al., 2000), the conditions in which this microbial community existed could not be determined. To further constrain environmental conditions in the different layers of an active chimney, Kormas et al. (2006) used mineralogical composition as a proxy for *in situ* temperature. The authors determined that archaeal diversity in each layer was in general agreement with predicted conditions, and thus that physical and chemical gradients probably had an important effect on the distribution of microbial populations. Nevertheless, temperature predictions for the deepest layer that contain Archaea could not be precisely constrained (60-240°C), and included conditions that should be lethal to all known microorganisms.

The controversial isolation of an archaeon that could grow at 121°C in laboratory conditions (Kashefi and Lovley, 2003) has set a new theoretical limit for microbial growth in active chimneys. However, several deep-sea vent microorganisms have shown the ability to grow at temperatures above their known maximum when cultured under high pressures (Baross and Deming, 1993). Many Archaea also possess cellular mechanisms that allow them to survive

several hours under lethal temperatures (Trent et al., 1990; 1994; Holden and Baross, 1993). Thus, some microorganisms might have the capacity to temporarily occupy zones of active chimneys that would be considered inhospitable for any life form. Given the large fluctuations observed in environmental conditions within some chimney sections (Tivey et al., 2002), this aptitude might be used as part of a strategy to gain an advantage during the initial colonization of newly available surfaces. Similarly, Archaea that can survive lethal H_2S concentrations and pHs (Lloyd et al., 2005; Edgcomb et al., 2007) might also get a competitive advantage. Although there is a potential for some cell movement during sample collection (Kormas et al., 2006), the detection of microorganisms in deep portions of active chimneys should therefore not necessarily be viewed as an artifact. Nonetheless, laboratory studies of microbial tolerance to extreme conditions do not provide clear indications of threshold values that prevent microbial colonization inside sulfide deposits.

As expected, chimneys formed by vastly different hydrothermal fluids harbor distinct microbial communities. The most striking of these cases resides in the atypical communities associated with chimneys of Lost City, Rainbow, and Logatchev vent fields. At these sites, the subsurface oxidation of ultramafic rocks (serpentinization) leads to emissions of hydrogen- and methane-rich fluids (Donval et al., 1997; Kelley et al., 2001; Perner et al., 2007). Consequently, microbial communities are dominated by methanogens, methane-oxidizers, and

hydrogen-oxidizers (Lopez-Garcia et al., 2003; Schrenk et al., 2004; Kelley et al., 2005; Brazelton et al., 2006; Perner et al., 2007).

Based on these observations, Nakagawa et al. (2005c) assessed the effect of fluid phase separation (i.e. boiling, Butterfield et al., 1997) on microbial community composition in chimneys of the same vent field. The authors reported that *Epsilonproteobacteria* appeared associated with fluids that are representative of most mid-ocean ridges, whereas *Deltaproteobacteria* seemed associated with gas-depleted fluids, and methanogens with gas-enriched fluids. Although these conclusions might be biased by the large differences in fluid temperatures between the samples analyzed in this study (311°C for the standard fluids, 247°C for the gas-depleted, and 70°C for the gas-enriched), Perner et al. (2007) noted a similar trend between bacterial diversity and hydrogen concentration in fluids from Logatchev. In the latter study, fluids containing 5.9 mmol H_2/kg carried different potential hydrogen-oxidizing lineages (*Aquificales*, *Epsilonproteobacteria* group D) than fluids containing 2.2 mmol H_2/kg (*Epsilonproteobacteria* group A and G). Despite the evidence showing that differences in end-member fluid chemistry can be linked to microbial diversity, it is likely that additional factors influencing thermal and chemical gradients in chimneys can affect microbial communities. For example, the dominance of hyperthermophilic anaerobes in a 4-day old black smoker (McCliment et al. 2006) suggests the presence high temperatures and anaerobic conditions throughout the chimney wall. On the other hand, microbial communities in mature chimneys

formed by comparable fluids include lineages associated with low temperatures and aerobic conditions (Schrenk et al., 2003; Hoek et al., 2003; Pagé et al., 2004; Kormas et al., 2006). Although models suggest that these observations can result from variations in fluid mixing styles (Tivey, 2004), a direct relationship between differences in thermal/chemical gradients and microbial diversity has not been demonstrated yet.

1.2.2. Subseafloor and Seawater Input

In 1983, Baross et al. reported the growth of mixed microbial cultures from samples of ≥300°C hydrothermal fluids. The microorganisms could produce CH_4, CO, and H_2 at 100°C, which led the authors to hypothesize that a large subsurface microbial biosphere could be partly responsible for the presence of these gases in hydrothermal fluids. Although controversial, the subsequent recovery of DNA from fluids of various temperatures (~180-360°C, Straube et al., 1990; Deming and Baross, 1993) and isolation of hyperthermophilic Archaea from low temperature fluids (~5-20°C, Summit and Baross, 1998; 2001) supported the hypothesis. It was subsequently demonstrated that hydrothermal fluids can carry diverse archaeal and bacterial communities comprising thousands of different 16S rRNA gene sequences (Takai et al., 2004b; Nakagawa et al., 2005c; Sogin et al., 2006; Huber et al., 2007). The magnitude of subsurface biospheres remains

unclear, but microorganisms have been detected up to 128.9 m below the seafloor at PACMANUS (Kimura et al., 2003).

The presence of microorganisms in conduits of chimneys discharging high temperature fluids (250°C and 311°C, Takai et al., 2001; Nakagawa et al., 2005c) suggests that newly formed sulfide deposits might be 'inoculated' by subseafloor microbial biospheres. Hence, some of the microorganisms collected in the deepest layers of active chimneys might not be significant participants in the biogeochemical cycles of the structures. Comparisons of overall cell abundances (i.e. DAPI-stained cells) to abundances of highly active cells (i.e. FISH-stained microorganisms) confirm that large proportions of the communities in these chimney layers are inactive or very slowly growing (Schrenk et al., 2003). If this input constitutes a significant factor in the formation of microbial communities in black smokers, variations in the diversity of microorganisms carried by fluids (Huber et al., 2002; 2003) might explain some differences between chimneys. This situation is also true for 'contamination' from bottom ocean seawater, which contains some microorganisms (e.g. *Crenarchaeota* marine group I, Massana et al., 2000, Karner et al., 2001) that are frequently detected on the surface of chimneys discharging high temperature fluids (e.g. Takai and Horikoshi, 1999; Schrenk et al., 2003; Takai et al., 2004b). However, the recent isolation of a thermophilic member of the *Crenarchaeota* marine group I (Candidatus *Nitrosocaldus yellowstonii*, de la Torre et al., 2008) suggests that microhabitats might be available for these microorganisms in active sulfide deposits.

1.2.3. Biological Interactions

Recent studies of microbial succession have considerably improved our understanding of population interactions in aquatic microbial communities (e.g. Jackson et al., 2001; Jackson, 2003; Schmidt et al., 2007). However, the remote nature of deep-sea hydrothermal vents has limited the possibilities of *in situ* experimentation, and thus left investigators with few means of assessing such interactions. In 2000, Reysenbach et al. reported on the microbial diversity associated with a biofilm collected with an *in situ* incubation chamber deployed for 5 days over 112˚C fluids. The community included multiple order-level lineages of Archaea (e.g. Marine *Crenarchaeota* group I, *Thermococcales*, *Archaeoglobales*), but the heterotrophic order *Thermococcales* largely dominated the archaeal 16S rRNA gene library (71% of the clones). In comparison, the archaeal community in a biofilm collected after a 2-day deployment was dominated by phylotypes related to the autotrophic order *Methanococcales* (80% of the clones) (Reysenbach et al., 2002). These results lead the authors to hypothesize that products of autotrophic microbial activity might favor the growth of heterotrophic populations over time. Although heterotrophs have been detected as early colonizers at temperatures below 65˚C (Nercessian et al., 2003; McCliment et al., 2006), the presence of a microbial community dominated by chemolithoautotrophic Archaea (*Ignicoccus* spp.) in a 4-day old chimney (McCliment et al., 2006) also suggests that pioneer colonizers of newly formed high temperature chimneys are autotrophic. So far, these studies constitute the

only evidence suggesting that autogenic microbial succession (i.e. driven by processes that are internal to the community) occurs at deep-sea vents.

Among other biological factors that may shape microbial communities in active chimneys, viruses infecting deep-sea vent microorganisms (Geslin et al., 2003) should be responsible for temporal modulations in the abundance of some populations (as in Ortmann et al., 2002 and Breitbart et al., 2004); the presence of *Alvinellids* on structures should favor microbial populations that are adapted to live with these polychaetes (Haddad et al., 1995; Cary et al., 1997; Alain et al., 2002; Pagé et al., 2004); contamination of chimney samples by seawater microorganisms might be more frequent at vent sites where high biological activity leads to high concentrations of planktonic microbes (Takai et al., 2004a).

1.3. Context of Work

1.3.1. Historical Perspective on Use of SSU rRNA Genes to Describe Microbial Communities

The use of small subunit (SSU) rRNAs (16S and 18S) as a taxonomic tool in microbiology was pioneered by the research of Dr. Carl R. Woese and his colleagues. In 1977, Fox et al. and Woese et al. successively described the evolutionary relationships between SSU rRNAs belonging to 10 species of methanogens, and 13 species of microorganisms representing the three domains of life (now recognized as Bacteria, Archaea, Eukarya). Although the approach was not accepted rapidly by the scientific community (see Woese, 2007), SSU

rRNAs were eventually recognized as the best molecular evolution clocks because of their appropriate size, ubiquitous distribution, relatively slow evolution, and apparent freedom of horizontal gene transfer artifacts (Woese, 1987).

Understanding that many microorganisms could not be readily brought to pure culture, Dr. Norman R. Pace and his colleagues started to explore Nature's microbial diversity using the SSU rRNA approach in the mid-1980s. Taking advantage of advances in nucleic acid sequencing and recombinant DNA, they successfully isolated, sequenced, and analyzed 16S rRNA genes from many environments, including hot springs of Yellowstone National Park (e.g. Olsen et al., 1986; Pace et al., 1986) and ocean waters in the Sargasso Sea (Giovanonni et al., 1990). Techniques involved in these studies were then optimized and streamlined, and the sequencing and phylogenetic analysis of 16S rRNA genes became a widespread approach in microbial ecology.

1.3.2. Limitations of the 16S rRNA Gene Approach

As it relies on a PCR step, the procedure most commonly used in surveys of environmental 16S rRNA genes (based on Olsen et al., 1986) is vulnerable to a series of problems that can alter assessments of microbial community composition and structure. These problems can be classified in two groups: the formation of sequence artifacts (chimeras) and the biases altering the ratios of PCR products.

At least 5% of all 16S rRNA gene sequences available in GenBank are chimeras (Ashelford et al., 2005). Using a model community composed of

bacterial species belonging to different lineages (*Alpha-*, *Beta-*, *Gammaproteobacteria*, Gram-positive Bacteria), Qiu et al. (2001) observed that frequencies of chimera formation by Taq DNA polymerase errors and/or heteroduplex formation during PCR varied with the use of different enzymes (Z-Taq, LA-Taq, AmpliTaq), but remained between 2.5 and 8.7%. However, chimera frequencies can be minimized by lowering template concentration and the number of PCR cycles, by increasing the elongation time in PCR reactions (Qiu et al., 2001), and by reporting microbial diversity at a sequence similarity of 99% or less (Acinas et al., 2005). A series of programs have also been designed to identify chimeric 16S rRNA sequences and remove these artifacts from datasets (e.g. Huber et al., 2004; Ashelford et al., 2005).

Biases altering the ratios of PCR products have not been studied as exhaustively as processes generating chimeras. In 1998, Polz and Cavanaugh reported that template to product ratios can be skewed by the preferential amplification of sequences with GC-rich priming sites in mixtures containing various templates. Results obtained by Suzuki et al. (1998) also suggest that amplification of high concentration templates can be inhibited by the self-annealing of PCR products. Although these biases appear minimized by the lowering of annealing temperature (Sipos et al., 2007) and decreases in template concentration (Qiu et al., 2001), differences in genomic 16S rRNA gene copy numbers between different microbial lineages remain a major hurdle to the accurate environmental assessment of microbial community composition and

structure (Acinas et al., 2004). However, future advances in microbial genomics will likely provide enough information to accurately predict cell numbers based on the identity and abundance of 16S rRNA genes detected in specific samples.

1.3.3. Dissertation Hypotheses

Despite numerous potential biases associated with PCR-based surveys of 16S rRNA genes, this approach constitutes a powerful tool that has revealed crucial information on the microbiology of many environments (see review by Pace, 1997). For this reason, the research presented in this document was built on comparisons of microbial communities described by 16S rRNA gene analyses and the assessment to various parameters reflecting in situ conditions (temperature, mineralogy, chemistry). The goal of these studies was to test the general hypothesis that microbial communities change with differing environmental and biological factors in hydrothermal vent chimneys (see Fig. 1). However, it is important to note that microbial communities are described in terms of gene presence and abundance, and not in terms of direct microorganism observation.

1.3.3.1. Guaymas Basin Project

Samples of hydrothermal chimneys were collected at Guaymas Basin in order to:

1- determine whether the distribution of microorganisms in active chimneys is influenced by in situ temperature,

2- describe the microbial communities associated with young and mature chimneys from the same vent field.

As reported by Schrenk et al. (2003) and Kormas et al. (2006), microbial populations are not distributed evenly across the walls of active high temperature chimneys. Although this distribution appears linked in part to thermal gradients, combined assessments of microbial diversity and measurements of *in situ* temperatures have never been conducted on the same deposit. Furthermore, the culture of an Archaeon apparently capable of growth at 121°C (Kashefi and Lovley, 2003) sets a new value for the highest temperature at which microorganisms should be detected inside high temperature chimneys. On this basis, two hypotheses were tested (see Chapter 2):

1- microbial putative temperature preferences (i.e. from minimum to maximum temperature for growth) increase with depth and in situ temperature within chimney walls,
2- microorganisms are not present at temperatures above 121°C.

As reported by Reysenbach et al. (2002) and McCliment et al. (2006), microbial communities colonizing surfaces exposed to high temperature hydrothermal fluids for short periods of time are characterized by low diversities and dominated by autotrophic Archaea. In contrast, studies of microbial

communities associated with mature chimneys invariably report the important presence of lineages characterized by heterotrophic physiologies (see section 1.1.2.1.). On this basis, a third hypothesis was tested (see Chapter 2):

3- microbial communities in young chimneys are dominated by autotrophs, whereas communities in mature chimneys are dominated by heterotrophs.

1.3.3.2. Juan de Fuca Ridge Project

This project was designed to build on results previously obtained from Guaymas Basin chimneys. In order to determine whether evidence of microbial succession could also be observed at Main Endeavour Vent Field, this project's goal was to:

1- describe the microbial communities associated with young and mature chimneys from the same vent field.

The relative age of the deposits was determined by comparing their composition to models of mineral changes occurring during chimney growth (Haymon, 1983; Goldfarb et al., 1983). This approach can highlight the effects of biological activity on microbial communities, but also provide some indications of the links between chimney mineralogy, fluid mixing style, in situ conditions, and microbial community composition and structure. On this basis, one hypothesis was tested (see Chapter 3):

1- microbial communities in young chimneys are dominated by autotrophs, whereas communities in mature chimneys are dominated by heterotrophs.

1.3.3.3. Lau Basin Project

Samples of hydrothermal chimneys were collected at Lau Basin in order to:

1- describe microbial communities associated with mature chimneys formed by different high temperature fluids.

Large differences in fluid chemistry appear to have an influence on the composition of microbial communities supported by hydrothermal fluids. For example, unique vent fields like Lost City harbor specific microbial communities (e.g. Schrenk et al., 2004; Brazelton et al., 2006). However, the impacts of differences in fluid chemistry within and between vent fields of the same hydrothermal systems are not well demonstrated. Nakagawa et al. (2005) and Perner et al. (2007) suggest that variability in gas concentrations may modify microbial community composition on active deposits of the same system. On this basis, one hypothesis were tested (see Chapter 4):

1- mature high temperature chimneys formed by hydrothermal fluids with different chemical characteristics harbor different microbial communities.

CHAPTER 2: Temporal and Spatial Archaeal Colonization of Hydrothermal Vent Deposits (Pagé, A, Tivey, MK, Stakes, DS, and Reysenbach, AL (2008) *Environmental Microbiology* **10:**874-884)

2.1. Abstract

Thermocouple arrays were deployed on two deep-sea hydrothermal vents at Guaymas Basin (27°0.5'N, 111°24.5'W) in order to measure *in situ* temperatures at which microorganisms colonize the associated mineral deposits. Intact sections of three structures that formed around the arrays were collected after 4- and 72-day deployments (named BM4, BM72, and TS72). Archaeal diversity associated with discrete subsamples collected across each deposit was determined by PCR amplification of 16S rRNA genes. Spatial differences in archaeal diversity were observed in all deposits and appeared related to *in situ* temperature. In BM4, no 16S rRNA genes were detected beyond about 1.5 cm within the sample (>200°C). Phylotypes detected on the outside of this deposit belong to taxonomic groups containing mesophiles and (hyper)thermophiles, whereas only putative hyperthermophiles were detected 1.5 cm inside the structure (~110°C). In contrast, the more moderate thermal gradient recorded across TS72 was associated with a deeper colonization (2 to 3 cm inside the deposit) of putative hyperthermophilic phylotypes. Although our study does not provide a precise assessment of the highest temperature for the existence of microbial habitats inside the deposits, archaeal 16S rRNA genes were detected directly next to thermocouples that measured 110°C (*Methanocaldococcus* spp. in BM4) and

116°C (*Desulfurococcaceae* in TS72). The successive array deployments conducted at the BM site also revealed compositional differences in archaeal communities associated with immature (BM4) and mature chimneys (BM72) formed by the same fluids. These differences suggest a temporal transition in the primary carbon sources used by the archaeal communities, with potential CO_2/H_2 methanogens prevalent in BM4 being replaced by potential methylotroph or acetoclastic methanogens and heterotrophs in BM72. This study is the first direct assessment of *in situ* conditions experienced by microorganisms inhabiting actively forming hydrothermal deposits at different stages of structure development.

2.2. Introduction

Deep-sea hydrothermal vent deposits are formed by mineral precipitation occurring as hot, reduced, and metal enriched fluids are emitted on the seafloor. The walls of these structures form permeable mineral matrices, and thus allow a gradual mixing between flowing hydrothermal fluids and surrounding seawater (Haymon 1983; Goldfarb et al., 1983). Steep physical gradients and multiple chemical disequilibria created during this process provide habitats for chemolithoautotrophic microorganisms, which harvest available energy through several oxidation-reduction reactions under a wide range of temperatures (McCollom and Shock, 1997; Tivey, 2004). This primary production subsequently supports a diversity of heterotrophs, and gives rise to diverse

microbial communities (Karl, 1995; Jannasch, 1995; Reysenbach et al., 2002; Miroshnichenko, 2004; Miroshnichenko and Bonch-Osmolovskaya, 2006).

The study of environmental constraints on microbial colonization of active deposits has thus far been limited by the lack of combined assessments of *in situ* conditions and microbial distribution across single structures. Steep thermal gradients have been documented across newly formed chimneys (Tivey et al., 1990; Tivey et al., 2002) and predicted across mature chimneys (Tivey and McDuff, 1990; Tivey, 1995). These results suggest that temperature preferences of resident microorganisms should increase from seawater-dominated environments (outer deposits) to hydrothermal fluid-dominated environments (inner deposits). Furthermore, relatively stable microbial habitats are probably restricted to mineral layers where temperature fluctuations may be equal or slightly above the highest temperatures reported for the growth of deep-sea hydrothermal microorganisms. Spatial surveys of microbial 16S rRNA genes have generally reported the presence of mesophilic and slightly thermophilic phylotypes on outer chimney surfaces (e.g. *Epsilonproteobacteria*, *Crenarchaeota* Marine Group I), and thermophilic phylotypes in deeper mineral layers (e.g. *Archaeoglobales*, *Thermococcales*, *Methanococcales*) (Takai et al., 2001; Schrenk et al., 2003; Hoek et al., 2003; Pagé et al., 2005; Nakagawa et al., 2005c). A recent study, which reported a similar trend in the distribution of archaeal 16S rRNA genes, provided estimates of *in situ* temperatures for different chimney layers based on measured interior and exterior temperatures, porosity, and

mineralogical composition (Kormas et al., 2006). Nevertheless, the effect of *in situ* temperature on microbial distribution across high temperature deposits remains poorly described, and the highest temperature allowing a stable colonization of active structures remains unknown.

The characteristics of microbial habitats available across actively forming high temperature deposits are also likely to undergo temporal changes as *in situ* environmental conditions change. For example, the thermal conductivity and permeability of chimney walls are modified as young mineral matrices dominated by anhydrite evolve towards mature matrices formed of complex mineral assemblages (Haymon, 1983; Goldfarb et al., 1983; Peter and Scott, 1988; Tivey and McDuff, 1990; Tivey, 1995). A modeling study of diffusive/advective transport of mass and heat demonstrated that such evolution should lead to modifications of thermal gradients and fluid mixing styles, and thus affect the availability and spatial distribution of microbial habitats (Tivey, 2004). As suggested by Reysenbach et al. (2000; 2002), temporal changes in microbial community composition, from autotrophy to heterotrophy, could result from a progressive accumulation of organic material produced by local microbial activity. Additionally, McCliment et al. (2006) recently reported that autotrophic sulfur reducing hyperthermophiles (i.e. *Ignicoccus*) dominated archaeal communities in structures collected after 72 to 92 hours of growth. In contrast, most assessments of 16S rRNA genes associated with mature structures report a higher diversity that includes mesophilic to hyperthermophilic autotrophs and

heterotrophs (Takai et al., 2001; Schrenk et al., 2003; Hoek et al., 2003; Nakagawa et al., 2005c; Pagé et al., 2004; Kormas et al., 2006).

Our study was designed to determine the effects of *in situ* temperature and thermal gradients on the spatial distribution of microorganisms across high temperature deposits, and to compare the microbial diversity in chimneys that developed on the same vent for different periods of time. To achieve these goals, we deployed thermocouple arrays mounted on titanium scaffolds over venting fluids (300°C) at two sites on the seafloor within Guaymas Basin, Mexico. The characteristics of high temperature fluids emitted at this vent field are influenced by the presence of a thick layer (100-500 m) of organic- and carbonate-rich sediments (2-4% organic matter, 10-15% $CaCO_3$) overlying the spreading axis, and the passage of fluids through this layer is likely accompanied by precipitation of sulfide-forming metals (Fe, Mn, Cu, Zn, Co, Cd) in the subsurface and the dissolution of calcium carbonate contained in sediments (Von Damm et al., 1985). These reactions lead to increases in fluid pH (around 6.0), alkalinity, and to a decrease in the highest temperature of fluids emitted on the seafloor ($\leq$325°C) (Von Damm et al., 1985). As a result, mineral deposits formed by these fluids are composed mostly of carbonates (Peter and Scott, 1988), and thus are not as fragile as those composed dominantly of anhydrite ($CaSO_4$) and fine-grained sulfide minerals. Guaymas Basin was chosen for our study due to the latter characteristic, which facilitated the recovery of material that precipitated around thermocouple arrays. Here, we describe three deposits collected after array deployments over

high temperature fluids, report on the microbial 16S rRNA gene diversity and spatial distribution within the structures, and compare microbial diversity to *in situ* temperature data.

2.3. Experimental Procedures

Thermocouple arrays

Thermocouple arrays consisted of eight ~50 cm long Ti-sheathed 1/8th inch O.D. type-J thermocouples, 4 per sensor module, that were mounted on an open Ti frame. The frame allowed the array of sensing ends of the thermocouples to be placed over the vent orifice while the two sensor modules, containing electronics, remained at a safe distance from hot fluid. Each sensor module monitored signals from the reference junctions of 4 thermocouples and a reference thermistor that were set in epoxy within an 8-cm long, 2.9 cm diameter plastic tube to protect the wires and electronic components from seawater. Paired Cu wires connected these components, via a 40 cm long cable, to an analog-to-digital converter and micro-processor residing in the small Ti pressure housed sensor module. The thermocouples were scanned once every 5 min, with data transmitted via an inductively coupled link (ICL) to a central data logger (16 Mbytes of storage) and two serial ports interfaced to ICL coils, located within a second, larger Ti pressure housing. Data recorded included calibration voltages, voltages of thermocouples and resistances of thermistors. Thermocouples were calibrated prior to the experiment at 100°C, 250°C, and 400°C. Thermocouple accuracy is ±2°C and

precision is ±0.3°C. Thermistor accuracy is ±0.1°C and precision is ±0.02°C. Use of the ICL coils made it possible to switch out thermocouple arrays without disturbing the central data logger. In addition, an ICL coil from the ROV was used to monitor temperatures during emplacement of the thermocouple arrays to ensure accurate placement of the thermocouples around the vent.

Array deployments and sample collection

On March 3, 2003, an actively venting mushroom shaped structure was knocked over at a vent site named Broken Mushroom (BM), and a thermocouple array was placed over the open orifice at what had been the "stem" of the mushroom with ROV Tiburon (for images of structures see http://www.mbari.org/expeditions/GOC/ logbook/Leg2/ Mar3.htm). Following the recovery of this array with a section of the chimney that grew around it (on March 7, 2003, sample was named BM4 for Broken Mushroom 4-day old), a second thermocouple array was placed against the base of the newly broken "stem". Also on March 7, a third thermocouple array was placed at Toadstool Vent (TS) over a small vent opening following removal of a small active chimney located on top and near the edge of a broad, low-lying mound. The second and third arrays were recovered 72-days later (on May 18, 2003, samples were named BM72 and TS72 respectively). All arrays and minerals precipitated around the instruments were brought to the surface in closed bioboxes to reduce contact with seawater during recovery.

Molecular analyses

On shipboard, subsamples (~1 g) were collected from different depths across the deposits (Fig. 4-6). Genomic DNA (gDNA) was immediately extracted using a Soil DNA Extraction Kit (MoBio laboratories, Carlsbad, CA) and stored at -80°C. Additional subsamples were stored at -80°C or at 4°C in ethanol and used for DNA extraction after returning to the laboratory.

Denaturing gradient gel electrophoresis (DGGE) was first chosen to rapidly analyze microbial 16S rRNA gene diversity associated with each subsample. To achieve this, fragments of 16S rRNA genes were amplified from the gDNA extracts using Archaea-specific primer 344FGC (Bano et al., 2004) and universal primer 519RP (5'-GWATTACCGCGGCKGCTG-3'). PCR mixes (50 µl final volume) contained 1X PCR buffer (2 mM $MgCl_2$, Promega, Madison, WI), 200 µM of each dNTP (Applied Biosystems, Foster City, CA), 200 nM of each forward and reverse primer (Invitrogen, San Diego, CA), 1 U of Taq polymerase (Promega), and 50 µg of BSA (Invitrogen) to prevent potential PCR inhibition by compounds co-extracted with the DNA. PCR conditions were as follows: 5 min at 94°C, 40 cycles of 30 sec at 94°C, 30 sec at 48°C, and 30 sec at 72°C, and a final extension of 7 min at 72°C. For BM4 and BM72 subsamples, a nested PCR approach using 1µl of PCR products obtained through amplifications of near full-length 16S rRNA genes was necessary to obtain positive amplifications (primers and conditions described in next paragraph). PCR products were sorted in DGGE gels (1X TAE: 40 mM Tris-acetate, 1 mM EDTA, pH 8.0, 6% acrylamide, 30% to

70% gradient of urea and formamide) ran in 1X TAE for 4h at 200V (DCODE apparatus, Bio-Rad Laboratories, Hercules, CA). Gels were then stained with SYBR green (BioWhittaker Molecular Applications, Rockland, ME) and visualized with a Typhoon 9200 phosphoimager (Molecular Dynamics). The DNA content of each band was quantified by measuring band intensity using ImageQuant 5.2 software (Molecular Dynamics). Bands in DGGE gels were stabbed with sterile pipette tips, eluted in 30 μl of 10 mM Tris (pH 8.0) for 10 min, and 5 μl of the eluted DNA solution were used for PCR reamplification using conditions described previously. Sequencing was performed with primer 519RP. Fifty, 10, and 21 sequences were obtained from BM4 (10 subsamples), BM72 (3 subsamples), and TS72 (4 subsamples) respectively. These sequences represented 20-80% (BM4), 25-86% (BM72), and 43-83% (TS72) of the bands identified for each subsample in the DGGE gels. Sequences were compared to the GenBank database using BLAST analyses (Altschul et al., 1990).

Near full-length 16S rRNA genes were then amplified from selected subsamples to obtain a phylogenetic affiliation of phylotypes identified in DGGE analyses and confirm the observed diversity. PCR was conducted on gDNA extracts with Archaea-specific primer 21F (DeLong, 1992) and universal primer 1492R (Weisburg et al., 1991). PCR mixes were as described previously. PCR conditions were as follows: 5 min at 94°C, 40 cycles of 30 sec at 94°C, 30 sec at 50°C, and 1.5 min at 72°C, followed by a final extension of 7 min at 72°C. PCR products of near full-length 16S rRNA genes were purified with MoBio's

SpinClean kit and cloned using Invitrogen's TOPO TA Cloning Kit following the directions of the manufacturer. Plasmids were purified from 2 ml cultures of transformed *E. coli* using the protocol described by Ng et al. (1996), and inserts PCR amplified with primers M13F and M13R. PCR mixes were as described previously. PCR conditions were 5 min at 94°C, followed by 30 cycles of 94°C for 1 min, 50°C for 1.5 min, 72°C for 2 min, and a final extension of 6 min at 72°C. A total of 40 clones were screened with the enzymes HimPII and MspI (New England Biolabs, Beverly, MA) for restriction fragment length polymorphism (RFLP) profiles. One clone representing each RFLP profile was fully sequenced using primers 21F, 515F (5'-GTGCCAGCMGCCGCGG TA-3'), 1100F (5'-GGCAACGAGCGMGACCC-3'), 1492R, 907R, and 519RP.

Phylogenetic analyses

Near full-length 16S rRNA gene sequences were assembled with ABI's auto assembler software (version 1.4.0), and checked for chimeras using Pin Tail (Ashelford et al., 2005). Assembled sequences were compiled with ARB (www.arb-home.de), and aligned based on results of BLAST analyses using ARB's FastAligner, followed by a manual alignment according to secondary structure. A maximum-likelihood tree was then constructed using ARB's version of fastdnaML, with unambiguous alignment positions (868 bp). Sequences obtained from DGGE bands were then added to the ML tree using ARB (parsimony analysis).

Nucleotide sequence accession numbers

The nucleotide sequences reported in this paper have been deposited in the GenBank database under accession numbers DQ925926 to DQ926006 (DGGE bands) and DQ925859 to DQ925866 (cloned 16S rRNA genes).

X ray diffraction (XRD), transmitted and reflected light petrography

Polished epoxy-impregnated thin sections were made from representative portions of each recovered chimney, and mineral identifications were made using reflected and transmitted light petrography and by scanning electron microscopy (SEM) coupled with electron diffraction spectra (EDS) analysis. Additional mineral identifications of very fine-grained materials were made using XRD. Cu-Fe sulfide grains (chalcopyrite or cubanite) were too small to identify optically and amounts too small to identify by XRD, so are not distinguished.

2.4. Results and Discussion

Temperature history and mineral composition of hydrothermal deposits

At the first thermocouple array placed over the Broken Mushroom vent (BM, 27°0.65'N, 111°24.4'W, 1998 m), thermocouple 3-2 (TC3-2) recorded temperatures in excess of 200°C within 6 hours of the 4-day array deployment, indicating that it had been enveloped by mineral precipitates and was no longer in contact with cold seawater (Fig. 3a). Within 18 hours of emplacement, consistent high temperatures (>275°C) measured by TC3-3 and TC3-4 indicating that these

thermocouples had also been encased. In contrast, TC3-1 (microbiological sampling location A, see Fig. 4) exhibited a more gradual increase in temperature, with fluctuations that suggest that the structure was porous and permeable, and that flow conditions varied during the 4 days (Fig. 3a). These fluctuations are best explained by variable advection of cold seawater into, or hot vent fluid out of, the chimney. Temperatures at TC4-1, TC4-2, TC4-3, and TC4-4 were 246°C, 297°C, 299°C, and 302°C at recovery, respectively. Temperatures were not recorded, however, throughout the deployment because the listening device, an inductively coupled link (ICL), was not in close enough proximity to the matching ICL loop.

At recovery (after 4 days of growth), all thermocouples recording temperatures of ≥297°C were ≥2 cm inside the outermost limit of the deposit, and at uniform temperature relative to TC3-1 (Figs. 3a and 4). The lower and variable temperature at TC3-1 indicates that there was a steep temperature gradient across the outer 1 to 1.5 cm of the chimney. The recovered section of chimney BM4 consisted of a mixture of anhydrite and calcite, with minor to trace amounts of aragonite, barite and clay, and trace amounts of pyrrhotite, wurtzite, and chalcopyrite or cubanite.

At the second thermocouple array deployed at BM (BM72), all but one of the thermocouples recorded stable temperatures of 298°C to 302°C within 2.5 hours of the 72-day array deployment, indicating that they were either within a channel of hot vent fluid, or enveloped within a protective barrier and very close to the channel of hot vent fluid (Fig. 3b). By 12 hours and 8 to 10 days, respectively,

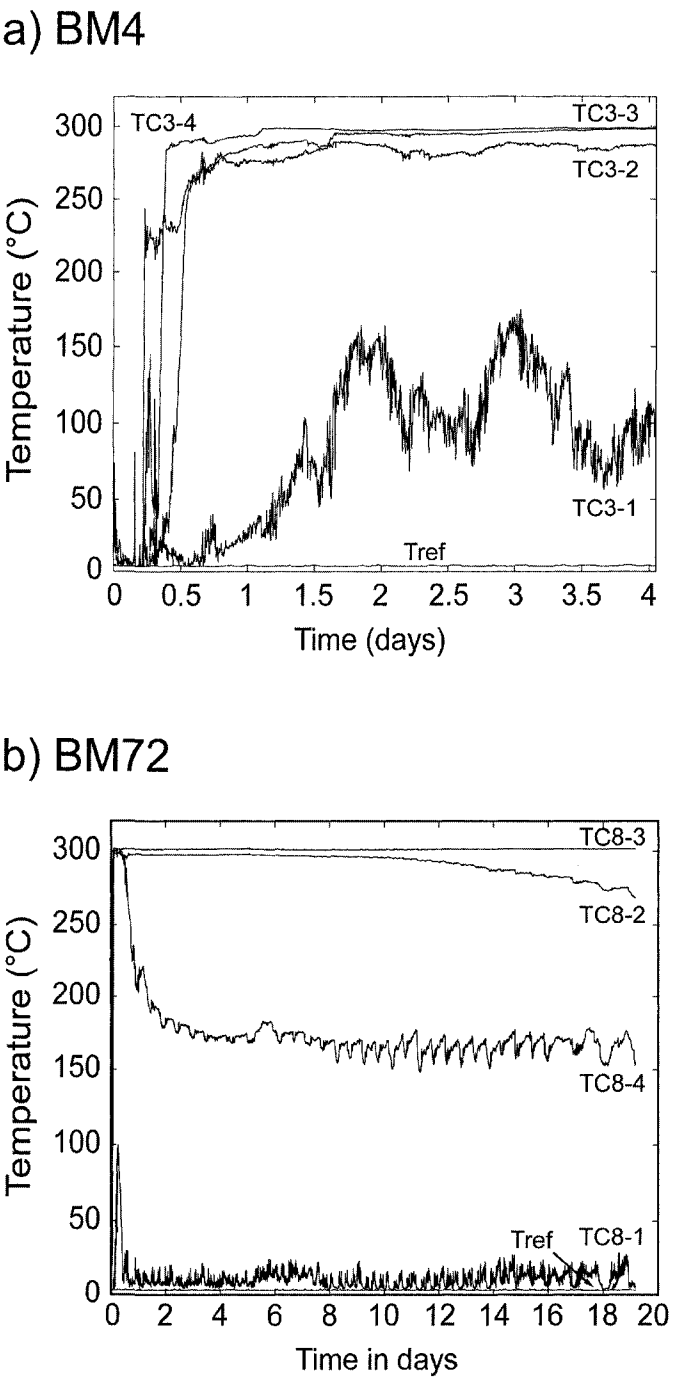

Figure 3. Temperature recorded within the deposits during the a) 4-day array deployment at Broken Mushroom (BM4), b) the first 19 days of the 72-day array deployment at Broken Mushroom (BM72). TC = thermocouple. Tref = reference thermistor.

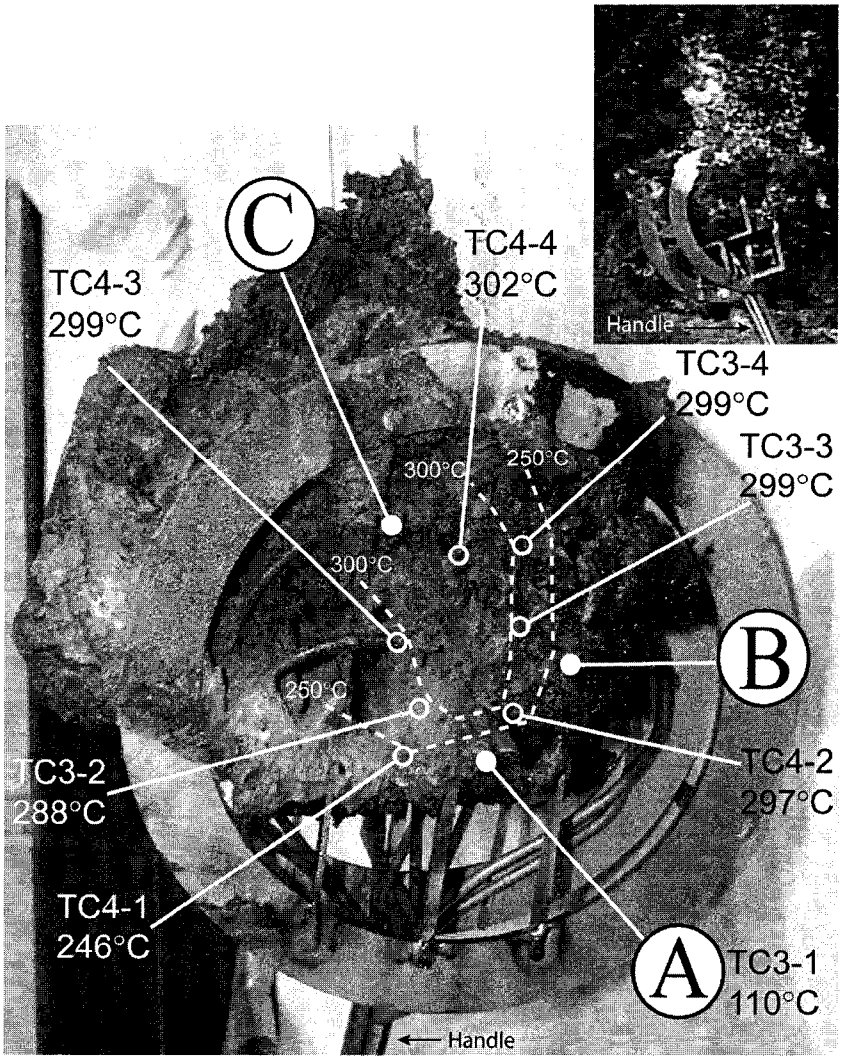

Figure 4. Chimney sample (showing the encased thermocouples exposed on the underside of the recovered thermocouple array) collected after the 4-day array deployment at Broken Mushroom (BM4). Temperatures indicated on the figure were recorded minutes before sample recovery. Letters indicate locations where subsamples were collected for microbial diversity analyses. Inset image shows the array in place on the seafloor prior to recovery. Open circles identify internal locations that cannot be seen on the figure. TC = thermocouple. Dashed lines show likely temperature contours within the sample, based on recorded temperatures at recovery.

TC8-4 (microbiological sampling location C) and TC8-2 were recording decreasing temperatures with time, consistent with having become incorporated into a thickening chimney wall. TC8-1 recorded a brief excursion up to 100°C early on, but then recorded low temperatures (<25°C) for the remainder of the 19 days (until a failure occurred in the sensor module), consistent with its location outside the chimney wall, where it was alternately bathed in cooler seawater and warmer fluids. Tidal periodicity in this record is consistent with wafting of warm and cold fluids against the chimney exterior in response to tidally driven bottom currents (as documented in Guaymas Basin (Little et al., 1988) and on the Cleft Segment (Tivey et al., 2002)). The within-structure ~12-hour periodic temperature variability (of 15°C to 25°C at TC8-4) was probably due to either advection of cold fluid through the walls in response to pressure changes caused by strong bottom currents, or variability in the temperature gradient within the wall caused by periodic temperature variability at the outer boundary, as was observed for a thermocouple encased within an active vent structure at the Cleft Segment (Tivey et al., 2002). While temperatures were not recorded after day 19, the records from the first 19 days documented a well-established mean temperature gradient across the wall, and it is likely that the mean temperatures at TC8-4 and TC8-2 continued to decrease gradually until the array was recovered. Given the established temperature gradient, outer parts (the outer few mm) of the deposit likely were not exposed to extremes in temperature after the first 12 hours. The recovered 1 to 2 cm-thick chimney wall section, BM72, was composed of calcite

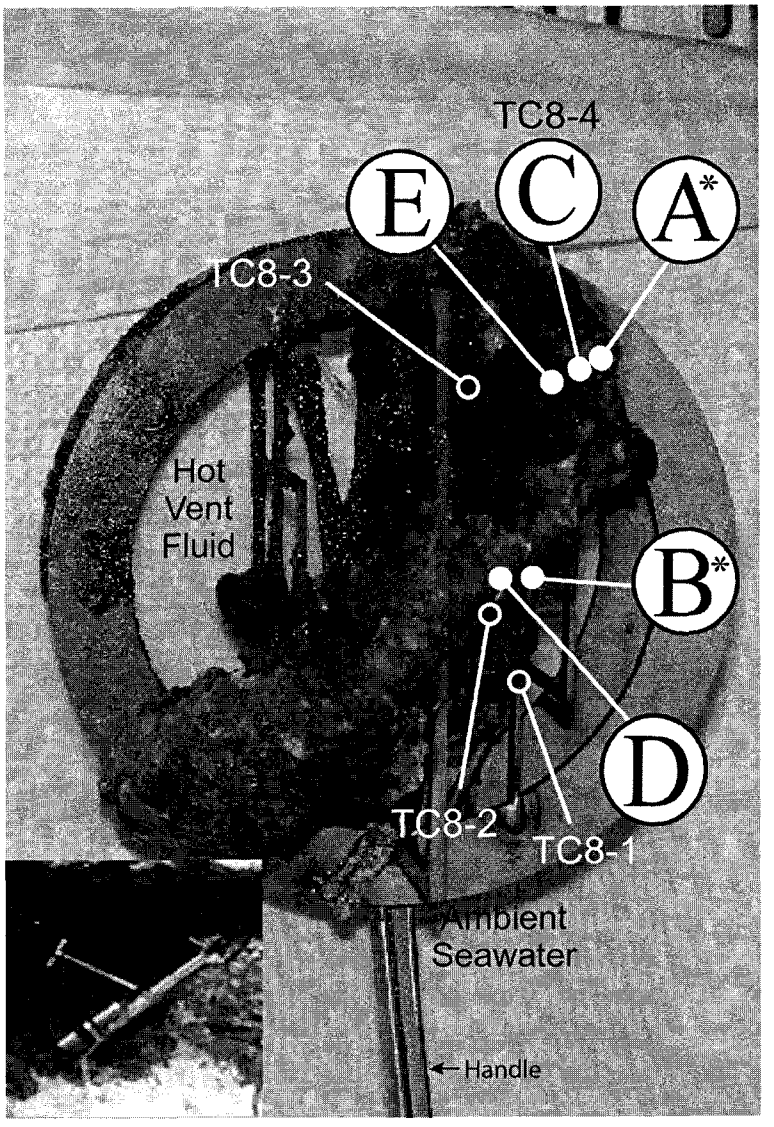

Figure 5. Chimney sample (a 2 to 3 cm thick wall separating hot vent fluid from ambient seawater) collected after the 72-day array deployment at Broken Mushroom (BM72). Inset image shows the array in place o the seafloor prior to recovery. Letters indicate locations where subsamples were collected for microbial diversity analyses. Open circles identify internal locations that cannot be seen on the figure. TC = thermocouple. Asterisks at A and B indicate locations where a clone library of near full-length archaeal 16S rRNA genes was built.

and anhydrite with lesser amounts of barite and clay, and trace amounts of sulfide minerals, including pyrrhotite, wurtzite, chalcopyrite and/or cubanite (Fig. 5). As described by Peter and Scott (1988), the initial wall was likely fairly porous and permeable, but as high temperature venting continued, calcite precipitated within interstices and as a replacement phase, decreasing the permeability and porosity of the wall.

At the thermocouple array deployed over the Toadstool vent (TS, 27°0.43'N, 111°24.56'W, 2001 m deep), temperatures were not successfully recorded during the 72-day deployment because of a failure of the ICLs to successfully transmit data to the datalogger. Temperatures immediately before recovery were recorded, and ranged from 116°C (at TC6-4, microbiological sampling location A) to 283°C (Fig. 6). The deposit that enclosed this array was a shallow dome composed dominantly of calcite with lesser amounts of barite, anhydrite, and trace amounts of pyrrhotite, wurtzite, and chalcopyrite or cubanite. A 3 cm diameter wide, 2 cm deep "cavern" with a "roof" of calcite was present inside this dome, and is interpreted here as a cavern that likely contained pooled hot vent fluid. Contours can be drawn using recorded temperatures (Fig. 6), with lowest recorded temperatures about 4-5 cm from the edge of the cavern and ~1 to 2 cm from the exterior of the structure. Given the lack of time-series temperature records from this array, information about temperatures over the 72-day deployment needed to be inferred based on the structure and composition of the deposit. The calcite-dominated dome that encased the array exhibited a low porosity. It likely formed

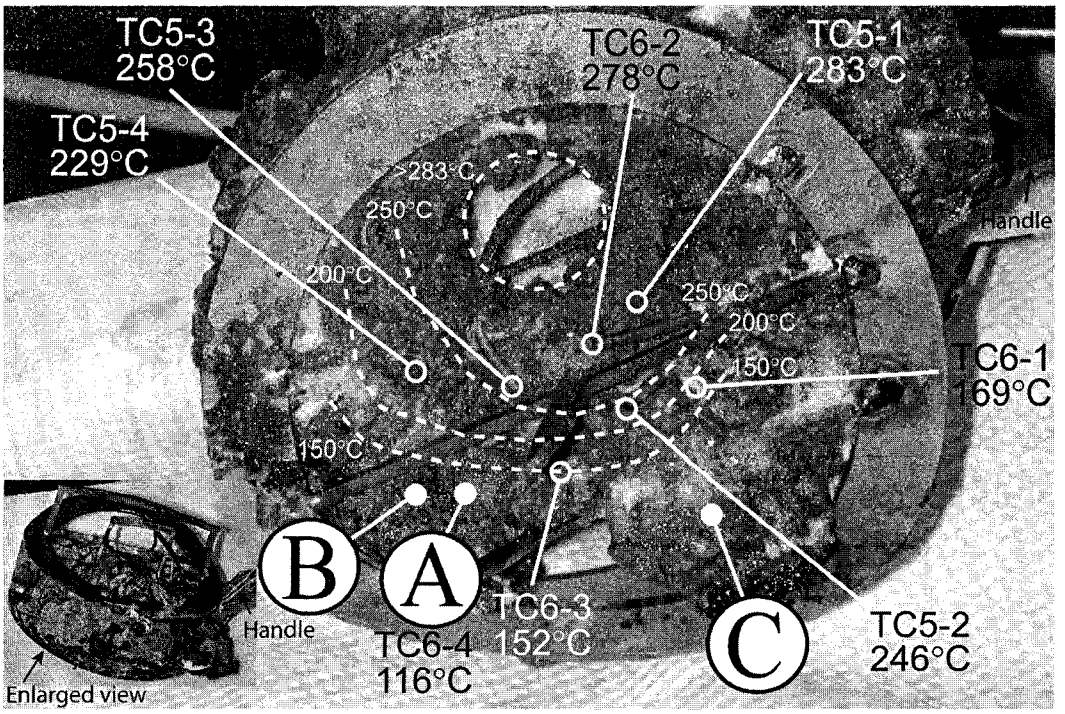

Figure 6. Chimney sample (showing the encased thermocouples exposed on the underside of the recovered thermocouple array) collected after the 72-day array deployment at Toadstool (TS72). Inset image shows the upper/outer domed surface of the sample within the encased array. Letters indicate locations where subsamples were collected for microbial diversity analyses. Open circles identify internal locations that cannot be seen on the figure. TC = thermocouple. Dashed lines show likely temperature contours within the sample, based on recorded temperatures at recovery.

as a more porous structure, with calcite precipitating within interstices and as a replacement phase, resulting in lower porosity and permeability over time (e.g., Peter and Scott, 1988). That the temperature distribution was zoned from higher temperatures near the cavern to lower temperatures near the exterior and furthest

from the cavern suggests that there was a fairly well established temperature gradient across the dome walls, with a steeper gradient across the "roof" above the pooled fluid (from >283°C to ambient across ~1 cm) and a shallower gradient across the base of the dome (shown in plan view in Fig. 6) from the >283°C interior pool out to the exterior of the dome (near subsamples A, B, and C) across 6 to 8 cm.

Temperature of microbial habitats within hydrothermal deposits

In situ temperatures appear to have had a strong influence on the microbial colonization of deposits collected at Guaymas Basin. Differences in temperature and potential temperature preferences of detected phylotypes coincided across BM4, BM72 and TS72. Although the failure to amplify bacterial (data not shown) or archaeal 16S rRNA genes from all subsamples examined should not be considered a proof of their absence, these locations were all characterized by temperatures that should prevent the growth of most microorganisms (Fig. 3-6).

In BM4, higher *in situ* temperatures corresponded to an apparent reduced range of available microbial habitats. Using DGGE to resolve 16S rRNA gene PCR products, archaeal phylotypes belonging to the hyperthermophilic genus *Methanocaldococcus*, thermophilic genus *Methanothermococcus*, and mesophilic *Crenarchaeota* marine group I (MGI) (Könneke et al., 2005) were detected in subsamples from the exterior 1 to 5 mm of the deposit (Fig. 7). Likewise, a clone library of 16S rRNA genes revealed the presence of *Methanocaldococcus* spp.

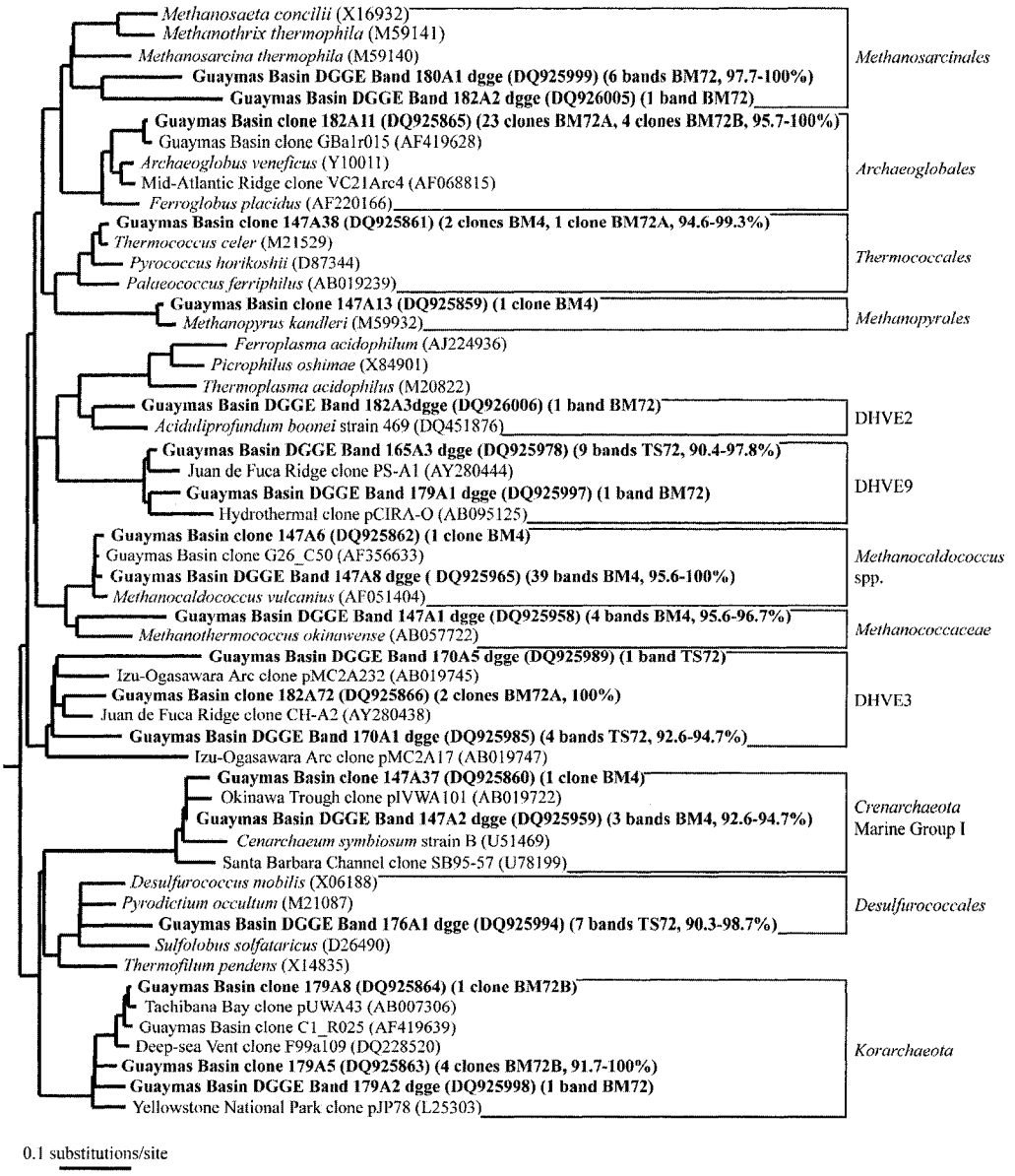

Figure 7. Phylogenetic relationship of archaeal 16S rRNA genes as determined by maximum-likelihood analysis. In parentheses, number of clones from each library represented by the phylotype, and percentages of similarity of the additional clones to this representative sequence (based on alignments performed with ARB and results by Moyer et al. (1996) for RFLP patterns). Outgroup formed of *Aquifex pyrophilus* (M83548) and *Thermotoga maritima* (M21774).

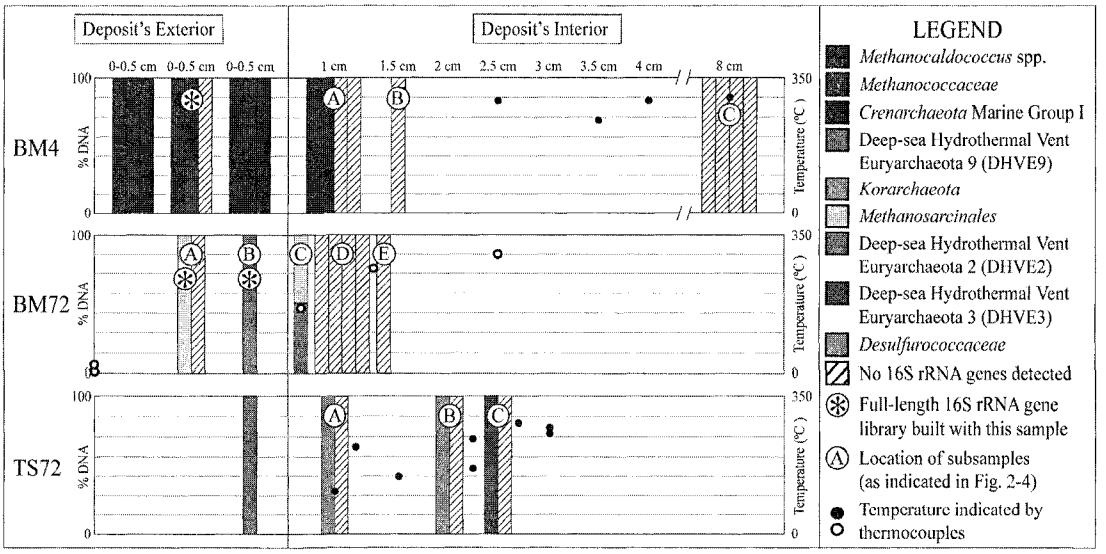

Figure 8. Schematic of sample locations and phylotypes from the three different deep-sea hydrothermal vent deposits from Guaymas Basin, Mexico. The phylotypes were detected through DGGE analysis of 16S rRNA gene fragments. Each bar represents a subsample taken at a specific location. In some subsamples, no 16S rRNA genes were detected (grey hatch) which is indicative of the microbial heterogeneity of colonization in these porous deposits or merely the limitations of our detection techniques. Percentages of DNA from different microbial groups in each subsample were evaluated through measurements of total DGGE band intensity (see experimental procedures). Circles represent temperature recorded *in situ* by thermocouples (minutes before recovery for BM4 and TS72 (closed circles), after 19 days of growth for BM72 (i.e. (open circles)). Circles represent temperature recorded *in situ* by thermocouples (minutes before recovery for BM4 and TS72 (closed circles), 19 days after deployment for BM72 (i.e. (open circles)). Note that, unless circles are directly within columns that indicate presence or absence of genetic material, the temperatures do not correlate directly with microbiological sampling location. For example, sub-samples B and C from TS72 were taken from locations 2 to

2.5 cm from the exterior but in areas likely at <150°C (see Fig. 6). In contrast, many of the thermocouple locations were also ~3 cm or less from the exterior of the sample, but at much higher temperatures because they were much closer to the inferred hot pool of fluid (see Fig. 6).

(95.6-100% sequence similarity to DGGE band sequences) and *Crenarchaeota* MGI (90.3-100% sequence similarity to DGGE band sequence) in this exterior layer. Phylotypes related to the hyperthermophiles *Methanopyrus kandleri* and *Thermococcus* species were also obtained from this library (Fig. 7). With the exception of the *Crenarchaeota* MGI, the potential temperature preferences of Archaea detected in this layer was consistent with the hypothesized steep thermal gradient across the outer 1 cm of BM4, based on *in situ* temperature measurements. Representatives of the *Crenarchaeota* MGI constitute dominant components of pelagic archaeal communities (Massana et al., 2000; Takai et al., 2004a), and were therefore probably not resident on BM4. In contrast with the archaeal diversity obtained from DGGE analyses of BM4's exterior, only potential hyperthermophiles (*Methanocaldococcus* phylotypes) were detected at ~1 to 1.5 cm within the deposit at TC3-1 (microbiological sampling location A, Fig. 8). Temperatures measured at this location were ~110°C at recovery, and >60°C for 2 days prior to recovery.

The correlation between temperature and detected microbial diversity was not as clear in the sample collected after the longer array deployment at BM (BM72),

as temperature records across this sample were limited to the first 19 days of the 72-day array deployment. Archaea detected from the outer 0 to 5 mm of BM72 included phylotypes with no characterized representatives (*Korarchaeota*, DHVE9) and *Methanosarcinales*, which have a range of growth temperature preferences (Fig. 8). Archaea detected from within the wall (microbiological sampling location C), where temperatures were likely higher and more stable than at the exterior (based on temperature patterns in days 0.5 to 19) included the thermoacidophilic lineage DHVE2 (Reysenbach et al., 2006) and *Methanosarcinales.*

Similar differences in the potential temperature preferences of phylotypes were detected across TS72. DGGE analyses revealed the presence of uncultivated DHVE9 phylotypes in the exterior 1 to 5 mm of the sample (Fig. 7). This clade was previously detected on a chimney colonized by hydrothermal vent polychaetes (Pagé et al., 2004), and co-occurred with *Epsilonproteobacteria* on TS72 (data not shown). Together with interpretations based on the zonation of temperature across the sample, these results suggest that temperature in this outer layer favored mesophiles and moderate thermophiles. In contrast, members of the hyperthermophilic *Desulfurococcaceae* and the uncultivated lineage DHVE3 (Takai and Horikoshi, 1999) were detected from locations 1 and 2 cm inside the structure (microbiological sampling locations A and B). The temperature at recovery for one of these locations (TC6-4, location A) was 116°C (Fig. 8).

These results confirm previous observations of spatial shifts in microbial community composition, which appeared linked to different temperatures but were not combined to direct measurements of *in situ* conditions. For example, all potential hyperthermophilic archaeal phylotypes (*Thermococcales*, *Archaeoglobales*, *Methanococcales*, *Thermoproteales*, *Korarchaeota*, *Desulfurococcales*) appeared to be more abundant in the internal layers than on the outer surfaces of high temperatures chimneys studied by Schrenk et al. (2003) and Kormas et al. (2006). Although our analyses do not provide evidence that organisms were actively growing and thriving in these samples, the combined assessment of *in situ* conditions and microbial distribution also gives estimates of the highest temperature at which 16S rRNA genes were detected. No 16S rRNA genes were amplified from subsamples located 1.5 to 2 cm (BM4-B) and 8 cm (BM4-C) inside BM4 (Fig. 4), or located >1 cm inside the wall of BM72 (Figs. 4 and 8), which may suggest that the temperatures at these sampling points were too high to support growth. As shown by the contours in Fig. 2, the sample BM4-C was at a depth within the chimney similar to where thermocouples measured 302°C; sample BM4-B was at a depth within the sample where temperatures were likely between ~150°C and 200°C. However, archaeal DNA was collected and PCR amplified from locations where temperature was 116°C in TS72 (microbiological sampling location A, Fig. 6) and 110°C in BM4 (microbiological sampling location A, Fig. 4). In comparison, Kormas et al. (2006) estimated, based on measured interior and exterior temperatures, porosity, and mineralogical

composition, that the deepest layer where archaeal 16S rRNA genes were detected within a chimney formed by 350°C fluids was at locations with *in situ* temperatures of between 60-240°C. Microbes detected in our Guaymas subsamples (*Methanocaldococcus* at 110°C and *Desulfurococcaceae* at 116°C) were likely exposed to lethal temperatures, as the highest temperature for growth of *Methanocaldococcus* cultures is 92°C (*M. fervens*, Jeanthon et al., 1999), and only one *Desulfurococcaceae* isolate appears to grow at 116°C (Kashefi and Lovley, 2003). These results support studies suggesting that microorganisms colonizing high temperature deposits can survive at temperatures above their known maximum temperature for growth under high pressures and constant supplies of carbon and energy sources (Miller et al., 1988; Marteinsson et al., 1997; Lloyd et al., 2005; Edgcomb et al., 2007). The large temperature fluctuations recorded within outer 1 centimeter of BM4 (Fig. 3) also suggests that stress response mechanisms allowing the survival of Archaea at lethal temperatures (Trent et al., 1990; 1994; Holden et al., 1993) are key to survival in this highly variable hydrothermal environment.

Spatial differences in the microbial community composition across actively forming hydrothermal deposits were first recognized by Hedrick et al. (1992). The spatial heterogeneity was attributed to an increased exposure to hydrothermal fluids inside the sulfide deposit, which would favor archaeal colonization over bacterial colonization. Similarly, shifts in microbial community composition across active structures were documented using 16S rRNA gene approaches

(Takai et al., 2001; Schrenk et al., 2003; Nakagawa et al., 2005c; Kormas et al., 2006) and fluorescent *in situ* hybridization (Harmsen et al., 1997a; b; Schrenk et al., 2003). Although spatial distribution of microorganisms across chimneys walls appears highly influenced by temperature differences and porosity, assessments of *in situ* conditions had only been limited to estimates based on temperatures measured at the chimney boundaries at the time of sampling, coupled with chimney mineralogy (Kormas et al., 2006) or single temperature records in the vicinity of the sampling locations (McCliment et al 2006). Thus, our study represents the first report that directly establishes a link between differences of *in situ* temperature and microbial community composition across hydrothermal deposits.

Temporal evolution of microbial habitats within growing hydrothermal deposits

Successive deployments of thermocouple arrays at the same vent (BM) allowed us to collect two chimneys formed by the same fluids, but representing two stages of growth (BM4 and BM72). At the time of sampling, high temperatures were likely present close to exterior parts of both structures (within ~0.5 cm), which is consistent with the detection of thermophilic and hyperthermophilic archaeal lineages in the samples (*Methanocaldococcus*, *Methanopyrus*, *Thermococcus*, *Korarchaeota*, *Methanosarcinales*, *Archaeoglobales*, and DHVE2 represented by "*Aciduloprofundum boonei*", Reysenbach et al., 2006). However, a clear difference was observed in the putative carbon requirements of phylotypes in each

deposit. Methanogens using CO_2 and H_2 (*Methanocaldococcus* spp. and *Methanococcaceae*), which appeared prevalent in the 4-day-old chimney, were not observed in the 72-day-old chimney. Instead, methylotrophic/acetoclastic methanogens and fermenters were present in BM72 (*Methanosarcinales, Korarchaeota, "Aciduliprofundales"*). These observations suggest that the successional shift in community structure may have been driven by an increased availability of organic carbon sources. Similar conclusions can be drawn from results obtained by Reysenbach et al. (2000, 2002) and McCliment et al. (2006) with deployments of artificial substratum over venting fluids. Additionally, analyses of 16S rRNA genes in biofilms collected at the Mid-Atlantic Ridge by Reysenbach et al. (2000; 2002) revealed a temporal shift from an archaeal community dominated by autotrophic methanogens after a 2-day deployment (80% of the 16S rRNA gene clones after 2 days) to a biofilm dominated by heterotrophs after a 5-day deployment (71% of the 16S rRNA genes belonged to *Thermococcales)*. Phylogenetic analyses of archaeal community composition in young chimneys (72-92 hours) formed by high temperature fluids (>140°C) on the East Pacific Rise also indicated that autotrophic Archaea (*Ignicoccus* spp.) constitute the first colonizers of newly formed hydrothermal chimneys (McCliment et al., 2006). Furthermore, the apparent absence of heterotrophs in BM4, despite the high concentrations of organic alteration products carried in fluids (Von Damm, 1985; Kawka and Simoneit, 1987; 1990; Bazylinski et al., 1988; Haberstroh and Karl, 1989; Martens, 1990; Seewald et al., 1998) support

previous observations suggesting that microbial succession in hydrothermal deposits are driven primarily by local *in situ* microbial activity (Reysenbach et al., 2000; 2002).

2.5. Conclusion

Deployments of thermocouple arrays provided *in situ* temperature measurements that could be linked to the spatial and temporal microbial colonization of high temperature mineral deposits. Our analyses suggest that microbial distribution and colonization depth are linked to *in situ* temperatures and characteristics of thermal gradients across the structures, and that some resident microorganisms can tolerate temperatures above their maximum temperature for growth. Differences observed in archaeal 16S rRNA genes collected in two chimneys recovered from the same vent further suggest that microbial community composition succession occurs between initial 'pioneer' communities dominated by autotrophs followed by communities dominated by a diversity of heterotrophs. Potential cell movement associated with advection of seawater during sampling (Kormas et al., 2006) renders precise locations of microbes in these porous deposits very difficult to determine. Nevertheless, our study provides a framework for array deployments at multiple sites, which would provide opportunities to explore patterns of microbial colonization using both microbiological and environmental condition data.

CHAPTER 3: Relationships between Microbial and Mineralogical Diversity in Deep-Sea Hydrothermal Vent Deposits

3.1. Abstract

Hydrothermal chimneys emitting high temperature fluids (>340°C) were analyzed by X-ray diffraction and 16S rRNA gene sequencing to compare microbial communities associated with deposits of different growth stages. Statistical comparisons demonstrated that microbial community composition and structure differs considerably between newly formed and mature chimneys. Only archaeal 16S rRNA genes could be amplified from a newly formed chimney composed mainly of anhydrite, whereas microbial communities associated with two mature sulfide deposits included Archaea and Bacteria. Comparisons of community structure using TreeClimber and SONS revealed that each chimney sample harbored a distinct microbial community, and that only small proportions of operational taxonomic units (OTUs) were shared between samples. All phylotypes associated with the newly formed chimney belonged to a single OTU closely related to *Archaeoglobus lithotrophicus*. In contrast, archaeal and bacterial OTU richness values predicted using a Chao1 estimate implemented by DOTUR were higher in mature deposits. Richness estimates ranged from 7-57 archaeal OTUs and 58-72 bacterial OTUs in the latter chimneys. Phylotypes identified from mature deposits belonged to a wide range of lineages (e.g. *Epsilonproteobacteria*, *Deltaproteobacteria*, DHVE5, *Thermococcales*). These observations suggest that chemolithoautotrophic Archaea initiate the colonization

of high temperature chimneys. A diversification of microbial communities subsequently occurs through the addition of Bacteria and heterotrophic Archaea. Our study provides new insights into the processes that accompany microbial succession in high temperature deep-sea hydrothermal vent chimneys.

3.2. Introduction

Describing the mechanisms that define microbial succession in the environment constitutes an important objective of microbial ecology. To isolate the effect of specific processes, numerous studies have been conducted on mixed cultures in laboratory settings (e.g. Raskin et al., 1996; Lebaron et al., 1999; Eilers et al., 2000; Allers et al., 2007). Although these experiments can recreate modulations in the abundance of some populations, results generated by this approach provide only limited information on naturally occurring succession. In the environment, temporal replacements of microbial populations are driven by a complex interplay between numerous internal (autogenic) and external (allogenic) processes. For example, microbial communities in alpine soils experience a combination of rapid autogenic microbial turnover and year-round seasonal succession linked to allogenic patterns of snow deposition and melt (Schmidt et al., 2007).

At deep-sea hydrothermal vents, biological succession is generally triggered by the formation of new vents following volcanic eruptions (Delaney et al., 1998; Shank et al., 1998). Surfaces that are exposed to hydrothermal fluids get rapidly coated with microbial biofilms, which could play a crucial role in the subsequent

settlement of megafauna (Taylor et al., 1996; Shank et al., 1998; Alain et al., 2004). Although very little is known about patterns of microbial succession in these extreme ecosystems, studies of microbial communities colonizing artificial substrates (Taylor et al., 1996; Reysenbach et al., 2000; 2002; Nercessian et al., 2003; Alain et al., 2004) and newly formed chimneys (McCliment et al., 2006; Pagé et al., 2008) reveal that pioneer populations are likely selected by the physical and chemical characteristics of hydrothermal fluids. Yet, the mechanisms governing the evolution of microbial communities past this initial colonization remain unclear.

As high temperature hydrothermal fluids (~300-400°C) mix with cold oxygenated seawater, minerals precipitate to form porous sulfide deposits ("chimneys") that are rapidly colonized by Bacteria and Archaea. These communities colonize the porous sections of chimney walls, where hydrothermal fluids mix with seawater to create steep physical and chemical gradients. Here, chemolithoautotrophs can exploit the chemical disequilibria of various oxidation-reduction reactions to gain energy and fix inorganic carbon (McCollom and Shock, 1997; Tivey, 2004). The intense metabolic activity occurring in zones of fluid mixing often leads to the formation of extensive biofilms on active chimneys (Chevaldonné and Godfroy, 1996; Schrenk et al., 2003; Hoek et al., 2003).

Local microbial activity and evolution of mineral matrices may help drive microbial succession in high temperature chimneys. As observed by McCliment et al. (2006) and Pagé et al. (2008), archaeal communities associated with nascent

high temperature chimneys (3- and 4-day old) appear largely dominated by autotrophic Archaea (e.g. *Ignicoccus* spp., *Methanocaldococcus* spp). When comparing these results with archaeal diversity in a 72-day old chimney (*Methanosarcinaceae*, '*Aciduliprofundales*', *Korarchaeota*), Pagé et al. (2008) concluded that a more diverse community of heterotrophs colonized the sulfide deposits after the initial autotrophic colonizers. A similar shift was observed with communities colonizing *in situ* growth chambers after 2 and 5-day deployments over hydrothermal fluids (*Methanococcales* and *Thermococcales*, respectively, Reysenbach et al., 2000; 2002). The organic carbon produced by the autotrophic populations probably support heterotrophic colonizers. In addition to this apparent autogenic succession, microbial communities might undergo allogenic succession driven by temporal changes in the mineralogical composition of growing chimneys (Haymon, 1983; Goldfarb et al., 1983). Furthermore, modeling of *in situ* conditions in chimney walls suggests that *in situ* gradients are significantly different across anhydrite-dominated (young) and sulfide-dominated (mature) mineral matrices, which would result in a diversification of available microbial microhabitats (Tivey, 1995; 2004).

Here we describe general patterns of microbial community composition and structure in three chimneys representing different stages of mineralization. This temporal evolution of mineralogy can be used as a proxy to explore temporal succession of microbial communities colonizing actively forming sulfide deposits. We show that the newly formed deposits are rapidly colonized by pioneering

sulfate reducing autotrophs, and then replaced with communities of higher species richness and metabolic diversity.

3.3. Materials and Methods

Sample collection

Sections of chimneys emitting high temperature fluids (>340°C) were collected during a 2005 oceanographic cruise to the Main Endeavour Vent Field (Juan de Fuca Ridge, 45°95'N, 129°11'W). The top of three structures growing on edifices Sully (SulX), Dante (DanX), and Smoke & Mirrors (SMX) were removed with the robotic arm of DSV *Alvin* (Woods Hole Oceanographic Institution). Chimney samples were brought to surface in closed bioboxes to reduce contact with seawater during submarine ascents. Once shipboard, samples were homogenized using a mortar and pestle, and either processed immediately for DNA extraction or stored at -80°C.

X-ray diffraction (XRD) analyses

To determine the relative age of each deposit, their mineralogy was compared to models of mineral composition changes occurring during chimney growth (Haymon, 1983; Goldfarb et al., 1983). Mineral identifications were conducted on 1 g aliquots of each chimney homogenate. Samples were air dried, crushed with a mortar and pestle, and sieved until they were entirely reduced to a <63 μm

fraction. XRD analyses were then conducted with a PHILIPS X-pert XRD Multi Purpose Diffractometer. This instrument uses Cu$K\alpha$ radiation at 40kV and 30mA. Samples were scanned from 5 to 75 °2Θ over 30 min. XRD tracings were analyzed with software X'Pert Graphics and Identity 1.2d (Phillips). Minerals were identified based on d spacing and peak intensity (JCPDS card #6-226 for anhydrite, #25-288 for chalcopyrite, #6-710 for Pyrite, #3-799 for Marcasite, #5-0492 for Wurtzite, #25-411 for pyrrothite).

DNA extractions

Genomic DNA was extracted from 1.5 g of each homogenate using MoBio Laboratories' Soil DNA Extraction Kit. DNA was quantified using Pico Green assays (Invitrogen) with a Stratagene MxPro 3005P QPCR instrument. Since DNA could not be detected in the first SulX extract (1.5 g), an additional extraction was conducted on 36.6 g of the homogenate using MoBio Laboratories' Mega Soil DNA Extraction Kit.

Quantification of 16S rRNA gene using QPCR

Archaeal and bacterial 16S rRNA genes were quantified in each DNA extract by real-time QPCR using QIAGEN's Quantitect SYBR green PCR kit and the Stratagene instrument described earlier. PCR conditions and primers were as described in Reysenbach et al. (2006) for amplifications of archaeal genes, and as described in Castillo et al. (2006) for amplifications of bacterial genes. The

specificity of amplifications was verified by the addition of dissociation curves at the end of each PCR reaction. Threshold fluorescence values were determined using adaptive baseline corrections and an amplification-based threshold calculation algorithm. Initial gene copy numbers in each sample were calculated based on the functions generated by plots of threshold cycles (Cts) against initial gene copy numbers for all points of the standard curves (average of triplicates for samples, triplicates or duplicates for standard curve points).

Construction of 16S rRNA gene libraries and sequencing

The archaeal and bacterial diversity associated with each chimney sample was assessed through cloning and sequencing of PCR-amplified 16S rRNA genes. PCR reactions contained primers 1492R (Weisburg et al., 1991) and 27F (DeLong, 1992) for bacterial gene amplifications, and 1492Raa (5'-ACGGHTAC CTTGTKWCGACTT-3') and 21Faa (5'-TCYSGTTGATCCTGCS-3') for archaeal gene amplifications. Primers for archaeal 16S rRNA gene amplifications were designed to encompass nanoarchaeal genes (Huber et al., 2002), and were verified against archaeal 16S rRNA gene alignment using ARB (http://www.arb-home.de). PCR conditions were as described in Pagé et al. (2008) for full-length amplifications, with the exception of primer concentration (400 nM), *Taq* DNA polymerase concentration (2U per reaction), and number of PCR cycles used (35 for bacterial amplifications).

PCR products were purified with MoBio Laboratories' Spin-Clean Kit, cloned and transformed with Invitrogen's TOPO TA Cloning Kit, and plasmids were isolated from 1.2 ml cultures of transformed *E. coli* as described in Pagé et al. (2008). Single sequencing reads (~600 bp) were obtained from 50 archaeal and bacterial clones per chimney sample (no bacterial sequences for SulX). Primer 1391R (5'-GACGGGCGGTGWGTRCAA-3') and 27F were used in archaeal and bacterial sequencing reactions respectively. Sequences were checked for chimeras using Pin Tail (Ashelford *et al.*, 2005). Nucleotide sequences are available through GenBank under the accession numbers EU559747 to EU559896 for Archaea and EU559897 to EU559996 for Bacteria.

Phylogenetic analyses

Bacterial and archaeal 16S rRNA gene sequences were assigned to taxonomic divisions on the basis of phylogenetic analyses. Sequences were compiled in ARB (www.arb-home.de) and aligned to reference sequences based on results of BLAST analyses using ARB's FastAligner. A manual refinement of the alignments was then conducted using secondary structure as a guide. Neighbor-joining trees were constructed with ARB, using only unambiguous alignment positions shared by all phylotypes (468 positions for Bacteria, 427 positions for Archaea).

Estimation of species richness and comparison of microbial community structures

Estimates of species richness in 16S rRNA gene libraries were conducted using DOTUR (Schloss et al., 2005). This program assigns 16S rRNA gene sequences to operational taxonomic units (OTUs) based on nucleotide similarity (i.e. percentages of nucleotide positions shared between sequences). In our study, similarities between each pair of sequences in a dataset were obtained by generating distance matrices from ARB alignments using a mask comprising nucleotide positions 183 to 539 (*E. coli* numbering) for Bacteria and 1102 to 1323 (*M. jannaschii* numbering) for Archaea. These gene segments were selected because sets of 25 OTUs (bacterial or archaeal) belonging to phylogenetic groups obtained in the study showed identical average phylogenetic distance between themselves over this gene segment as over the entire gene. Sequences were assigned to OTUs using the 'furthest neighbor' algorithm and the 97% similarity criterion (i.e. all sequences within an OTU are ≥97% similar to all other sequences in the group). With the observed OTU richness values and the number of sequences sampled in each library, richness estimates and 95% confidence intervals on estimate values were calculated by DOTUR based on the algorithm developed by Chao (1984).

Estimated proportions of shared OTUs between 16S rRNA gene libraries were obtained with SONS (Schloss et al., 2006b). This program implements incidence-based measures of community similarity. In our study, Sorenson similarity indices (S_{clas}) were generated based on estimated richness values generated by DOTUR

(Chao1 estimates using 97% similarity criterion for assignment of sequences to OTUs).

Comparisons of community structure (diversity and abundance of sequences in each lineage) were conducted between 16S rRNA gene libraries using TreeClimber (Schloss et al., 2006a). TreeClimber uses the parsimony test to determine the relatedness of communities. First, a composite phylogenetic tree in which each taxon (16S rRNA sequence) is assigned to a specific sample is built. The tree is then scored using Fitch's parsimony algorithm (i.e. a penalty if given to each external branch sharing taxa assigned to more than one sample). The score of the tree is finally ranked among scores of 9999 additional trees generated randomly. A rank within the 5% lowest score ($p < 0.05$) indicates a significant effect of the treatment between samples (i.e. communities are statistically different). Composite phylogenetic trees used in our analyses were built as described in the phylogenetic analyses section of the materials and methods.

3.4. Results

Chimney mineralogy

Each chimney sample differed in their bulk mineralogy (Fig. 9). SulX was composed of primarily anhydrite and chalcopyrite, typical of a relatively newly formed sulfide deposit. In addition to anhydrite and chalcopyrite, DanX also contained pyrite, pyrrhotite, and wurtzite. In contrast, only marcasite, pyrite, and wurtzite were detected in the sample from SMX.

Quantification of 16S rRNA genes

Consistent with DNA yields from each extract (Fig. 9), the highest number of 16S rRNA genes was measured in SMX, followed by DanX and SulX. Only archaeal 16S rRNA genes could be PCR-amplified from SulX, whereas Archaea and Bacteria were both detected in DanX and SMX. Nevertheless, QPCR analyses revealed that archaeal 16S rRNA genes outnumbered bacterial genes by approximately two orders of magnitude in the latter samples (Table II). Given that bacterial genomes generally contain higher numbers of 16S rRNA genes than archaeal genomes (Acinas et al. 2004), these results suggest that Archaea dominated the microbial communities in both cases. However, primers used in the analyses of archaeal 16S rRNA genes have been shown to successfully amplify different bacterial genes (e.g. *Aquificales*, *E. coli*, Ferrera, pers. comm.). Concentrations of archaeal 16S rRNA genes might therefore be overestimated due to unspecific amplifications. Nevertheless, the successful amplification of archaeal 16S rRNA genes was demonstrated by the presence of PCR products of expected sizes, the absence of unspecific amplification in non-template controls, unique peaks with high melting temperatures in dissociation curves, and the successful amplification and sequencing of archaeal 16S rRNA genes with different primer sets from the same genomic DNA. Concentrations of bacterial 16S rRNA genes that are orders of magnitudes lower than concentrations obtained with the unspecific archaeal primers further suggest that archaeal genes dominated the DNA extracts. Based on these observations, and the large variance

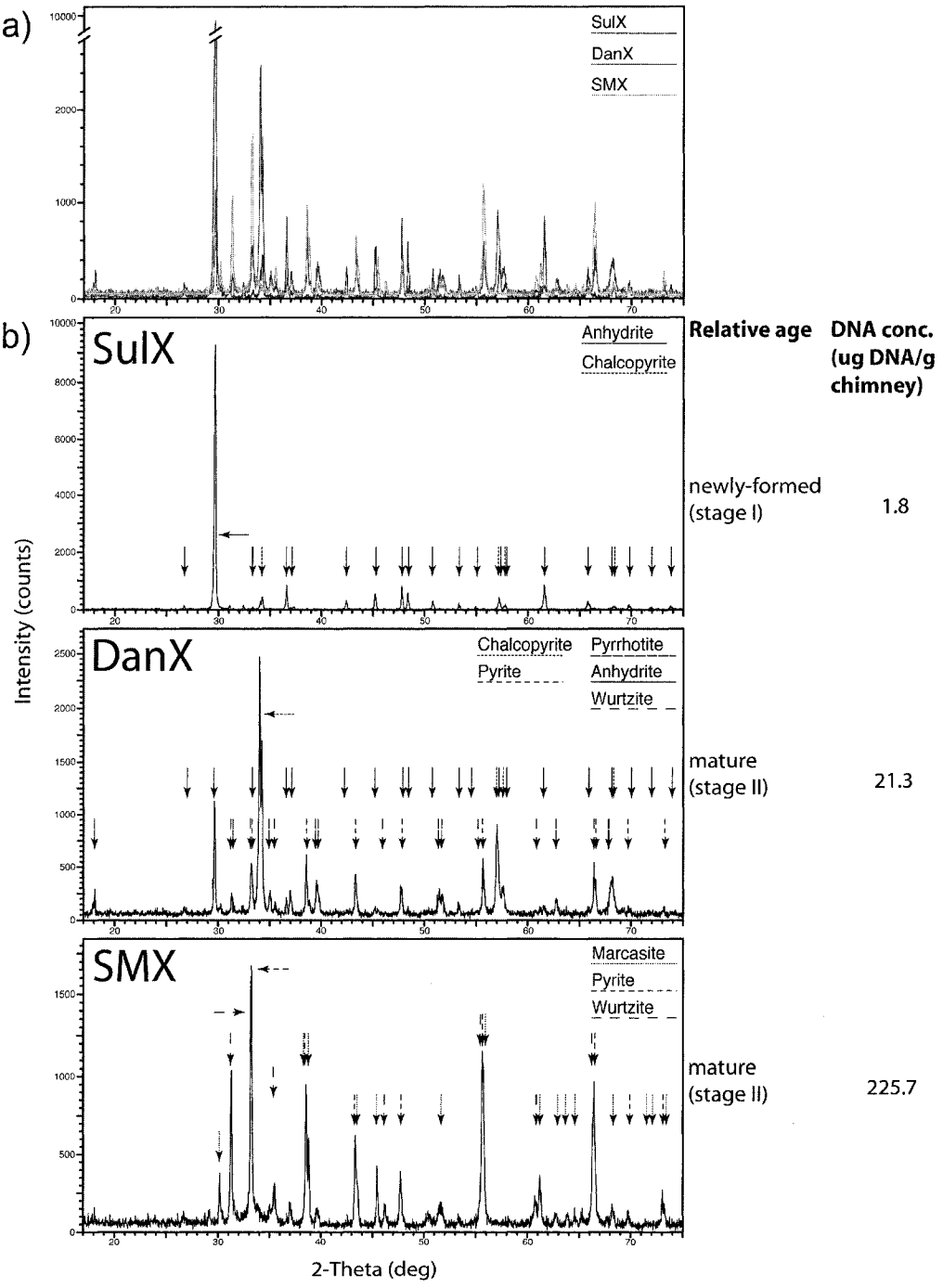

Figure 9. a) Relative crystallinity of chimney samples collected on the Juan de Fuca Ridge (as indicated by peak intensity measured during XRD analyses). b) Mineral composition and DNA content of the same chimneys. Minerals are identified by their

patterns of X-ray diffraction, on the basis of JCPDS cards #6-226 for anhydrite, 25-288 for chalcopyrite, 6-710 for pyrite, 25-411 for pyrrhotite, 5-0492 for wurtzite, and 3-799 for marcasite.

on the gene copy number averages, gene copy results presented here should not be considered as absolute, but rather seen as relative ratios between bacterial and archaeal genes.

Comparisons of microbial community structure and diversity between samples

Rarefaction analyses revealed low OTU diversities (97% distance for OTU definition) in archaeal 16S rRNA gene libraries built with DNA from SulX and DanX (data not shown). These observations were supported by estimates of OTU richness using a Chao1 model, which predicted low richness in both cases (Table II). Thus, our sampling effort (50 clones sequenced) was sufficient for estimates of richness using these libraries. Rarefaction analyses revealed that OTU diversity was not exhausted in DanX's bacterial library and SMX's archaeal and bacterial libraries (data not shown) and estimates of OTU richness were higher in the latter cases (Table II). As a result, the bacterial community associated with DanX and the archaeal and bacterial communities in SMX were only represented by dominant OTUs in the statistical analyses. Nevertheless, important differences in estimates of archaeal OTU richness indicate that each chimney harbored a structurally distinct archaeal community. This interpretation was supported by a

TreeClimber analysis indicating that archaeal 16S rRNA gene sequences were not distributed randomly between the libraries ($p = 0.002$). Furthermore, Sorenson estimates predicted that SulX and DanX shared 0% of their total combined archaeal OTUs, whereas only 6.3% were shared between SulX and SMX, and 5.6% between DanX and SMX. Despite a large overlap in the 95% confidence intervals of their respective OTU richness estimates (Table II), the structure of bacterial communities associated with DanX and SMX were also distinct (TreeClimber analysis $p = 0.006$). Sorenson estimates predicted that 19.1% of total combined bacterial OTUs were shared between the two libraries.

Table II. Quantifications of 16S rRNA genes in DNA extracts obtained from three chimneys of the Juan de Fuca Ridge.

Sample	Bacterial 16S rRNA genes (16S rRNA genes/g wet of chimney)[a]	Estimated bacterial OTU richness (95% confidence intervals)	Archaeal 16S rRNA genes (16S rRNA genes/g wet of chimney)[a]	Estimated archaeal OTU richness (95% confidence intervals)
SulX	-	-	*$3.09X10^5 \pm 5.35X10^5$	1 (1-1)
DanX	$1.33X10^6 \pm 1.21X10^6$ (0.88%)	72 (35-189)	$1.50X10^8 \pm 2.43X10^7$ (99.12%)	7 (5-20)
SMX	$4.29X10^7 \pm 9.35X10^6$ (0.37%)	58 (34-138)	$1.14X10^{10} \pm 1.33X10^9$ (99.63%)	57 (39-112)

[a]In parentheses, proportion of total 16S rRNA genes detected (bacterial and archaeal). Note that concentrations of archaeal genes might be overestimated due to potential unspecific PCR amplification.
*Only 1 of 3 PCR reactions provided a positive amplification.

In addition to these important differences in community structure, the phylogenetic analyses of 16S rRNA genes revealed significant differences in the composition of microbial communities in each chimney. Most notably, SulX appeared exclusively colonized by Archaea related to *Archaeoglobus lithotrophicus* (Fig. 10). Although DanX also contained three *Archaeoglobales* OTUs, this sample was also colonized by representatives of two other archaeal lineages, DHVE5 and *Crenarchaeota* MGI. *Epsilonproteobacteria* and *Deltaproteobacteria* of the family *Desulfobulbaceae* were also detected in this sample (Fig. 11). The SMX sample had an even higher microbial diversity with at least 10 orders detected. As in DanX, *Epsilonproteobacteria* and *Deltaproteobacteria* dominated the bacterial community associated with SMX. However, the distribution of bacterial 16S rRNA gene sequences was uneven in all lineages that were shared between the two samples. Four bacterial lineages were also represented only in DanX (*Gammaproteobacteria*, Candidate Division OD1) or SMX (*Epsilonproteobacteria* Group E, *Cytophaga-Flexibacter-Bacteroidetes*).

3.5. Discussion

The development of black smokers is characterized by a sharp mineralogical transition between the two recognized growth stages (Haymon, 1983; Goldfarb et al., 1983). With its major anhydrite component, SulX constituted the only immature deposit of our sample set. Due to a retrograde solubility (precipitation

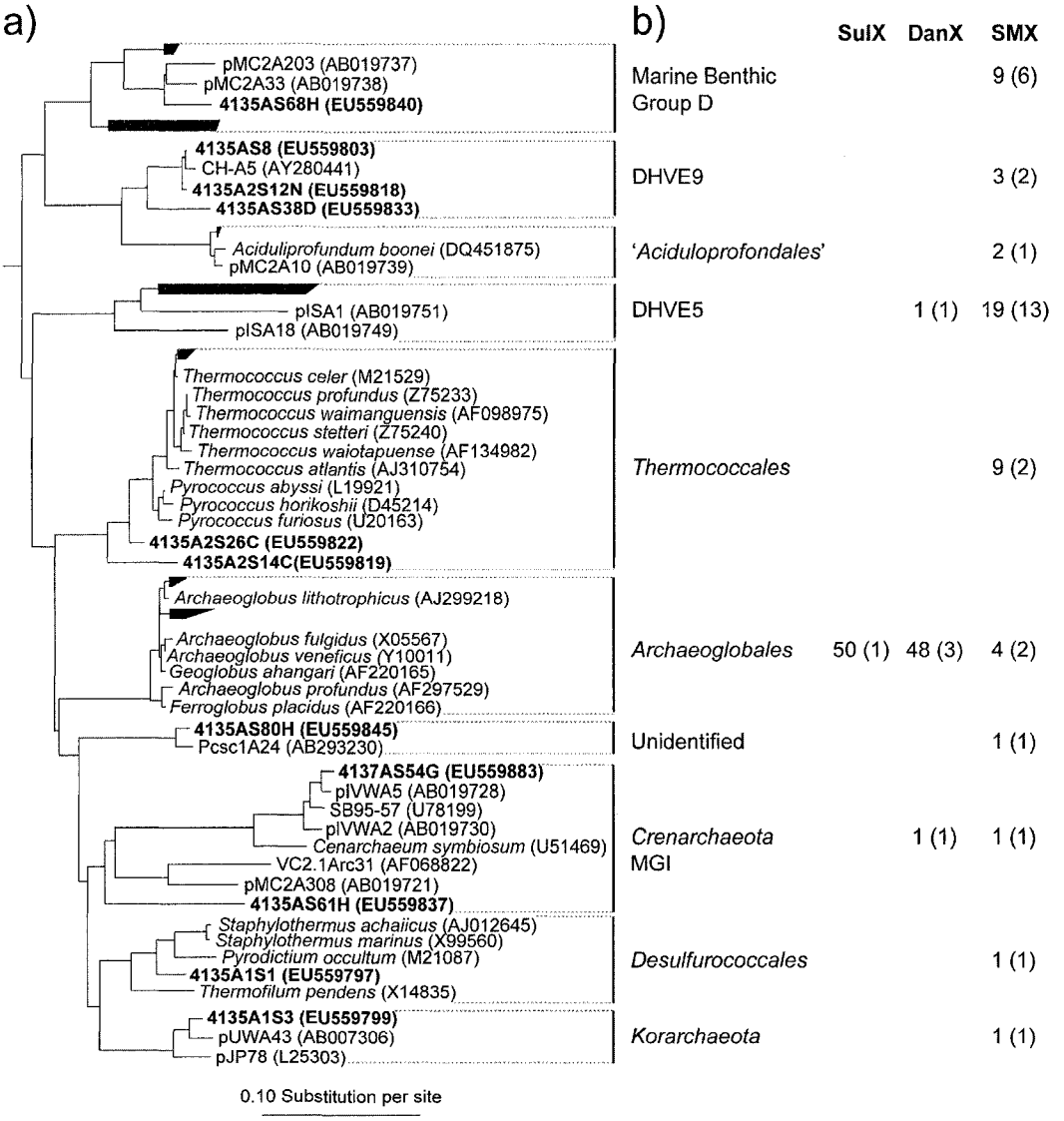

Figure 10. a) Phylogenetic relationship of archaeal 16S rRNA genes as determined by a neighbor-joining analysis. Wedges represent clades formed of sequences collected in this study, and their horizontal dimensions reflect the degree of divergence within the group. Outgroup formed of *Aquifex pyrophilus* (M83548) and *Thermotoga maritima* (M21774). b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries. In parentheses, the number of OTUs (97% similarity) observed in each division.

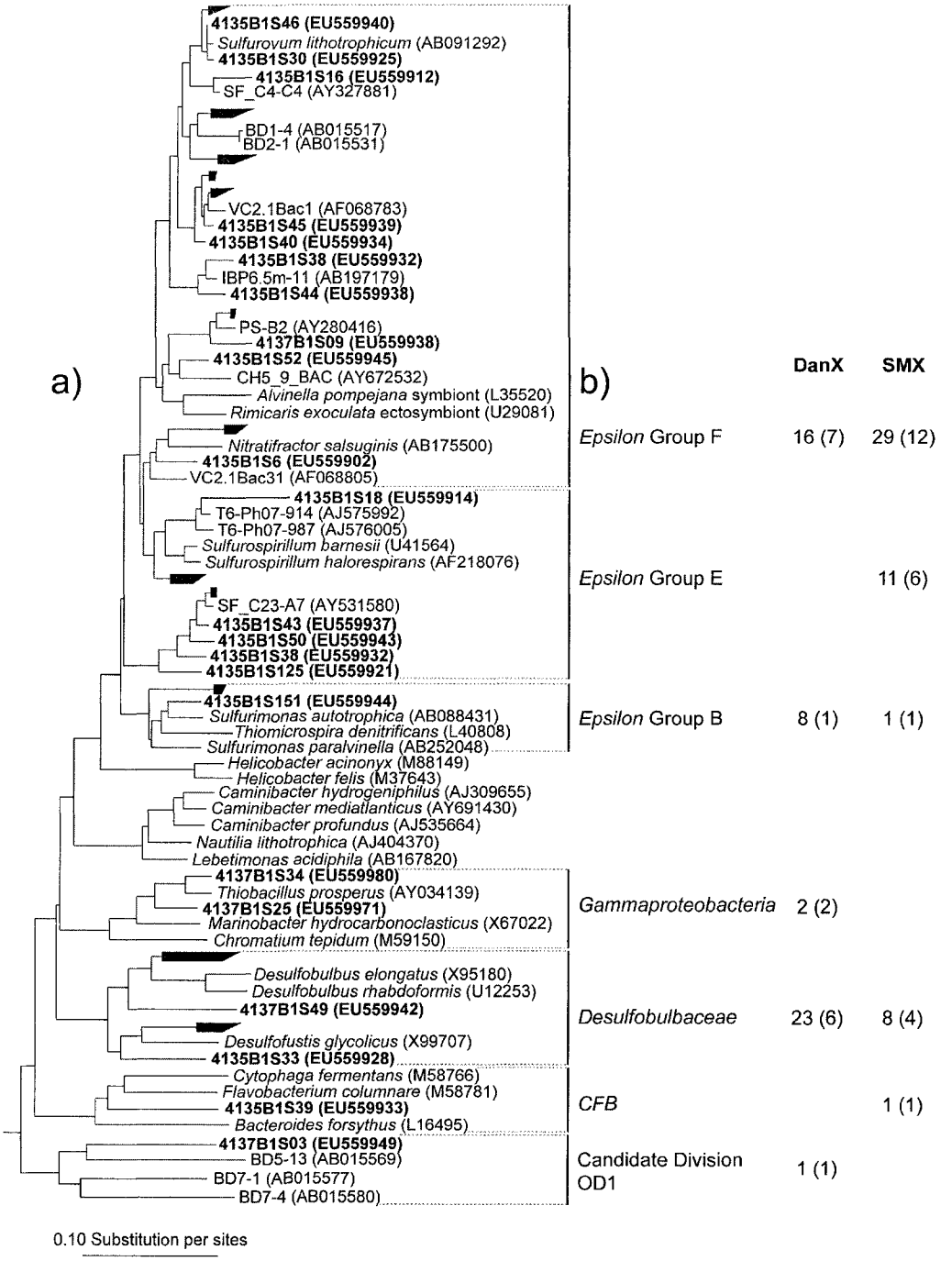

Figure 11. a) Phylogenetic relationship of bacterial 16S rRNA genes as determined by a neighbor-joining analysis. Wedges represent clades formed of sequences collected in this study, and their horizontal dimensions reflect the degree of divergence within the group.

Outgroup formed of *Methanocaldococcus jannaschii* (M59126) and *Thermococcus celer* (M21529). b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries. In parentheses, the number of OTUs (97% similarity) observed in each division.

above ~130°C), anhydrite is the first mineral to precipitate from high temperature mixtures of hydrothermal fluids and seawater. Thus, it is the main component of young chimneys (stage I) at sediment-starved mid-ocean ridges (Hannington et al., 1995). The relatively young age of SulX is confirmed by the identical mineral composition of a 14-day old chimney collected on a chemistry probe exposed to high temperature fluids (Pf14, data not shown).

Since attempts at extracting DNA from newly formed chimneys are often unfruitful (e.g. HiT in McCliment et al., 2006, Pf14 in this study), the recovery of PCR-amplifiable DNA from SulX provided a rare opportunity to describe the microbial community associated with one of these deposits. In contrast with new surfaces exposed to low temperature hydrothermal fluids, which are first colonized by various divisions of *Epsilonproteobacteria* (Taylor et al., 1996; Alain et al., 2004), Bacteria were not detected in SulX. A single population of Archaea closely related to the autotrophic sulfate-reducing hyperthermophile *Archaeoglobus lithotrophicus* (Boucher et al., 2001) was the only OTU detected in this newly formed deposit. To select for these microorganisms, the walls of SulX may have been characterized by high temperatures (above ~55°C) and

anoxic environments devoid of organic compounds that could support heterotrophic microorganisms.

Similar studies conducted at other vent fields have identified different archaeal divisions as the pioneer colonizers of newly formed high temperature chimneys. In 2006, McCliment et al. reported the detection of Archaea belonging to the sulfur-reducing genus *Ignicoccus* in a ~4 day-old chimney formed by >203˚C fluids. At Guaymas Basin, a 4 day-old chimney formed by 302˚C fluids was colonized by species of the methanogenic genus *Methanocaldococcus* (Pagé et al., 2008). These results suggest that variability in the characteristics of microbial niches available within newly formed chimneys might be linked to differences in the chemical composition of hydrothermal fluids between vent sites. For example, the presence of methanogens at Guaymas Basin is expected in light of the high concentrations of reduced gases dissolved in fluids from this vent field (Von Damm, 1995). Nevertheless, microbial colonization appeared constrained by the lack of oxygen, organic compounds, and high temperatures in all cases. These conditions might be created by the advection of vent fluid inside highly porous chimney walls. High temperatures measured *in situ* across several young chimneys (>100˚C approximately 1 cm inside the deposits, Tivey et al., 1990; 2002; Pagé et al., 2008) support this interpretation.

Microbial communities associated with mature high temperature chimneys

The presence of various sulfide minerals in DanX and SMX indicates that both chimneys had undergone the mineral transition leading to the second stage of black smoker growth. As primitive anhydrite walls partially isolate hydrothermal fluids from seawater, these minerals accumulate and gradually form distinct layers in stage II deposits (Haymon, 1983; Goldfarb et al., 1983). This transition is mineralogy, which is suggestive of an older age for these two deposits, was accompanied by a transition in microbial diversity.

The presence of bacterial populations was one of the main differences observed between microbial communities in young (SulX) and mature chimneys (DanX, SMX). Although SulX appeared devoid of Bacteria, DanX and SMX both contained populations related to mesophilic H_2S/S^0-oxidizers (*Epsilonproteobacteria* Group F, B), NO_3^--reducers (*Epsilonproteobacteria* Group F, B, *Desulfobulbaceae*), and/or SO_4^{2-}-reducers (*Desulfobulbaceae*). Thus, oxic and anoxic environments probably existed at temperatures below ~40°C in the two mature chimneys. Despite the large variability observed in the composition of deep-sea vent bacterial communities, these microbial phenotypes are hallmark features of mature high temperature chimneys (Schrenk et al., 2003; Hoek et al., 2003; Pagé et al., 2004; Nakagawa et al., 2005c, Kormas et al., 2006). Conditions supporting these communities might be created by the advection of seawater inside multi-layered chimney walls with reduced porosities. This mixing style allows the formation of large gradients of temperature and oxygen

concentration across chimneys, and positions the oxic/anoxic transition at temperatures around 21°C when vent fluids contain ~1.7 mmol of H_2 per kg of fluids (Tivey, 2004). Gradual temperature decreases measured in the outer portions of active chimneys (Pagé et al., 2008) also suggest that conditions tend to evolve in this direction over time.

Although each chimney harbored a different archaeal community, SMX contained the most diverse of the three. Despite some differences in composition and OTU richness, archaeal communities in SulX and DanX were both dominated by phylotypes related to the autotrophic sulfate-reducer *Archaeoglobus lithotrophicus*. Thus, the archaeal diversity associated with DanX appeared to constitute a transition between SulX and SMX. This observation is supported by the detection of anhydrite in DanX but not in SMX. Due to its reverse solubility, this mineral dissolves away from portions of chimney walls exposed to temperatures below ~130°C for extended periods of time. In sharp contrast, SMX contained a high archaeal OTU richness, and a large number of phylotypes belonging to lineages of putative heterotrophic microorganisms (*Thermococcales*, '*Aciduliprofundales*', *Korarchaeota*, Marine Benthic Group D). Based on relative ages suggested by the mineralogy of each chimney (SulX<DanX<SMX), these results suggest a temporal diversification of niches available to heterotrophic Archaea. Thus, the production of organic compounds by pioneer autotrophic populations might drive autogenic microbial succession in high temperature

chimneys, as suggested by Reysenbach (2000; 2002) and Pagé et al. (2008). Jackson et al. (2001) and Jackson (2003) indicate that this type of microbial succession involves three different stages of community development. The first succession stage is characterized by rapid increases in species richness, as multiple populations attempt to colonize the newly available surfaces under minimal competition. The gradual expansion of resident populations then enhances competition for resources, which leads to an intermediate stage where species richness decreases as superior competitors get established. Finally, biofilm growth and internal cycling of elements can lead to a diversification of available niches, and thus promote increases in species richness during the last succession stage. Although these successional stages were not observed in our analyses, our results suggest that a similar link between early and late colonizers might exist in high temperature chimneys.

3.6. Conclusion

Analyses of mineral composition provided a proxy to determine the relative ages of high temperature hydrothermal chimneys collected at Main Endeavour vent field (Juan de Fuca Ridge). Comparisons of microbial community composition and structure were then conducted to identify processes that could potentially drive microbial succession in these deposits. As demonstrated by estimates of OTU richness that are several orders of magnitude below values obtained by Huber et al. (2007), the sequencing of 50 16S rRNA genes per clone library

limited the comparisons to numerically dominant populations. Nevertheless, our results reveal that young and mature chimneys harbored distinct microbial communities. On the basis of microbial diversity associated with each sample type, we suggest that chimney growth might be accompanied by a combination of autogenic and allogenic microbial succession. Although bacterial colonization appears promoted by temporal changes to the mineralogy and configuration of chimney walls, archaeal succession appears linked to the internal cycling of organic compounds produced *in situ* by microbial activity. These results might be explained by the stratification of microbial habitats in active chimneys (McCollom and Shock, 1997; Tivey, 2004). As demonstrated by Schrenk et al. (2003), Archaea show a general preference for the deepest parts of chimney walls, where environmental conditions are least likely to fluctuate, whereas Bacteria prefer the more variable outer sections of chimney walls.

CHAPTER 4: Microbial Communities Associated with High Temperature Chimneys of the Eastern Lau Spreading Center and Valu Fa Ridge

4.1. Abstract

To test the relationships between characteristics of hydrothermal fluids (pH, chemistry) and microbial communities at deep-sea hydrothermal vents, we sampled high temperature chimneys along the Eastern Lau Spreading Center (ELSC) and Valu Fa Ridge (VFR). Due to the gradients in fluid chemistry occurring between basalt-hosted (ELSC) and andesite-hosted vent fields (VFR), this area constitutes an ideal location to test these interactions in sites that are in close proximity. As a preliminary effort, we assessed the microbial diversity associated with four high temperature chimneys collected at Kilo Moana (KM), Tow Cam (TC1 and TC2), and Mariner (MA) vent fields. Microbial community composition and structure were determined by sequencing of archaeal and bacterial 16S rRNA genes, and compared using DOTUR, TreeClimber, and SONS. These analyses revealed that each chimney harbored a distinct microbial community. KM, TC2, and MA, showed differences in proportions of dominant populations, but all included putative mesophilic sulfur compound-oxidizers (*Epsilonproteobacteria* group F, B), hyperthermophilic sulfate-reducers (*Archaeoglobales*), and hyperthermophilic heterotrophs (*Thermococcales*). Although these differences could not be linked to the chemistry of fluids discharged by each vent, the presence of some lineages (*Epsilonproteobacteria* group D, *Deltaproteobacteria*, *Geoglobus* spp.) may signal high H_2 and Fe(II)

concentrations in fluids of KM and MA respectively. In contrast, variability in fluid mixing styles appeared to explain the differences observed between microbial communities in TC1 and TC2. Although the two chimneys shared several mineralogical and fluid characteristics, TC1 was dominated by putative hyperthermophilic methanogens (*Methanocaldococcus* spp.) and thermophilic sulfur-reducers (*Epsilonproteobacteria* group D). Our study describes the microbial communities associated with chimneys collected from recently discovered hydrothermal vents sites, and highlights potential links between microbial habitats and environmental factors within these deposits.

4.2. Introduction

Hydrothermal systems occur in various geological settings, including mid-ocean ridges, island arcs, back-arc spreading axes, and hot spots. Due to considerable differences is crustal lithology and magma contribution, each of these systems possesses distinctive fluid characteristics (Seyfried and Mottl, 1995; Bach et al., 2004; Früh-Green et al., 2004). As fluid chemistry dictates the metabolic menu of primary producers (McCollom and Shock, 1997; Tivey, 2004; McCollom, 2007), the different systems also harbor distinct microbial communities. For example, the large quantities of H_2S discharged at basalt-hosted mid-ocean ridges (up to 12 mmol/kg of fluids, Von Damm, 1995) favor the development of communities largely dominated by sulfur compound-oxidizers of the division *Epsilonproteobacteria* (Takai et al., 2006b). In contrast, the H_2- and CH_4-rich

fluid (up to 19 and 4 mmol/kg of fluids respectively, Donval et al., 1997; Kelley et al., 2005; Proskurowski et al., 2006; Perner et al., 2007) created by serpentinization in ultramafic-hosted systems support microbial communities that include abundant populations of hydrogen- and methane-oxidizers (e.g. *Aquificales*, ANME-1, *Methylobacter* spp.) (Kelley et al., 2005; Brazelton et al., 2006; Perner et al., 2007).

Recent oceanographic cruises have discovered several new hydrothermal vent fields along the Eastern Lau Spreading Center (ELSC) and Valu Fa Ridge (VFR) (Micheal and Seewald, 2007). These back-arc spreading axes are located in one the most geologically complex areas on Earth, the Lau Basin (Smith et al., 2001; Zellmer and Taylor, 2001). Owing to a decreasing separation from the Tofua arc volcanic front, magma composition gradually changes from basaltic in the north portion of the ELSC to andesitic in the VFR (Pearce and Stern, 2006). Consequently, differences in source rock composition create geochemical gradients in hydrothermal fluids emitted along the spreading axes. This progression is characterized by increases in pH and decreases in H_2 and H_2S concentrations from Kilo Moana (north ELSC) to Tui Malila (south ELSC) (Seewald et al., 2005). South of Tui Malila, the composition of hydrothermal fluids undergoes a dramatic shift that appears influenced by the presence of a melt lens and small scale faulting allowing magmatic degassing (Micheal and Seewald, 2007). At Vai Lili and Mariner, hydrothermal fluids are characterized by low pH and high concentrations of CO_2 and metals (Fouquet et al., 1991; Seewald et al.,

2005). Since large variations in fluid chemistry are likely to affect the microbial diversity associated with different Lau Basin vent fields, this area constitutes an ideal location to assess the influence of large-scale geophysical processes on hydrothermal ecosystems.

Analyses of microbial diversity supported by ELSC and VFR hydrothermal systems have not been conducted yet. Based on gas and metal concentrations that are comparable to values measured at prototypical mid-ocean ridge sites (Von Damm, 1995), we hypothesize that fluids discharged in the northern portion of the ELSC should support abundant populations of sulfur compound oxidizers and reducers. These reactions are by far the most energetically profitable in typical mid-ocean ridge environments (McCollom and Shock, 1997). On the other hand, the exceptional characteristics of hydrothermal fluids emitted in the southern ELSC portion might allow the development of unique microbial communities.

Here, we describe patterns of microbial community composition and structure in four mature chimneys collected at three ELSC vent fields. Although a set of order-level lineages appeared to constitute the dominant portion of communities in three of the four chimneys, the presence of indicator microorganisms suggests that some link exists between middle-scale variations in vent fluid composition and microbial diversity. Furthermore, our results suggest that differences in fluid mixing style can also have considerable effects on microbial communities within active chimneys.

4.3. Materials and Methods

Sample collection and analyses

Hydrothermal vent chimneys discharging high temperature fluids (300-323°C) were collected during a 2005 oceanographic cruise that visited multiple active vent fields along the ELSC and VFR (TUIM05MV). The mineral deposits were recovered with the robotic arm of ROV *Jason II* (Woods Hole Oceanographic Institution), and brought to surface in closed bioboxes to reduce contact with seawater during ROV ascents. Samples were collected at Kilo Moana (20°2'S, 176°2'W, KM (J2-137-2-R1)), Tow Cam (20°3'S, 176°1'W, TC1 (J2-126-5-R1) and TC2 (J2-127-1-R1)), and Mariner (22°2'S, 176°6'W, MA (J2-130-1-R2). Once shipboard, external mineral layers were collected from each chimney (Fig. 12) and kept at -80°C until processed.

Further analyses were conducted as described in Chapter 2 (section 3.3). Procedures included extractions of genomic DNA (1-2 g for TC1, TC2, KM, and 12 g for MA), X-ray diffraction analyses, quantifications of archaeal and bacterial 16S rRNA genes, construction of 16S rRNA gene libraries and sequencing (GenBank accession numbers EU559997 to EU560196 for Archaea and EU560197 to EU560396 for Bacteria), phylogenetic analyses (610 positions for bacteria, 546 positions for archaea), estimation of species richness, and comparisons of microbial community structure.

4.4. Results

Characteristics of chimneys and end-member fluid chemistry

Chimneys sampled along the Eastern Lau Spreading Center fell in two general categories. The deposit sampled at the northernmost site, Kilo Moana (sample KM), was identified as a classic black smoker with a clear fluid conduit at the center of the deposit (Fig. 12a). The wall of the chimney was zoned, with a gradual transition from layers of chalcopyrite deep inside the sample (data not shown) to a layer dominated by pyrite, sphalerite, and marcasite on the surface (Fig. 13). Some iron-oxide patches were also observed on the outer surface of KM. Fluids emitted from this chimney (pH 3.6) contained high concentrations of H_2 and low concentrations of CH_4 (Table III, Seewald, Wheat, Mottl, pers. comm.).

In contrast, chimneys collected at Tow Cam were identified as 'white spires' (Fig. 12b, c), deposits that do not always form well-defined central conduits (Koski et al., 1984; Tivey, 1995) despite emitting high temperature fluids (318°C for TC1, 303°C for TC2, Table III). Although some differences were observed in minor mineral components, TC1 and TC2 were characterized by similar compositions. TC1 was predominantly composed of sphalerite and wurtzite, and contained traces of pyrite, marcasite, and chalcopyrite (data not shown). Numerous small iron sulfide-lined conduits were evident in this hard and relatively narrow and flat spire. The outer surface of the chimney was coated with a grey layer of sphalerite, marcasite, anhydrite, and pyrite (Fig. 13), and was

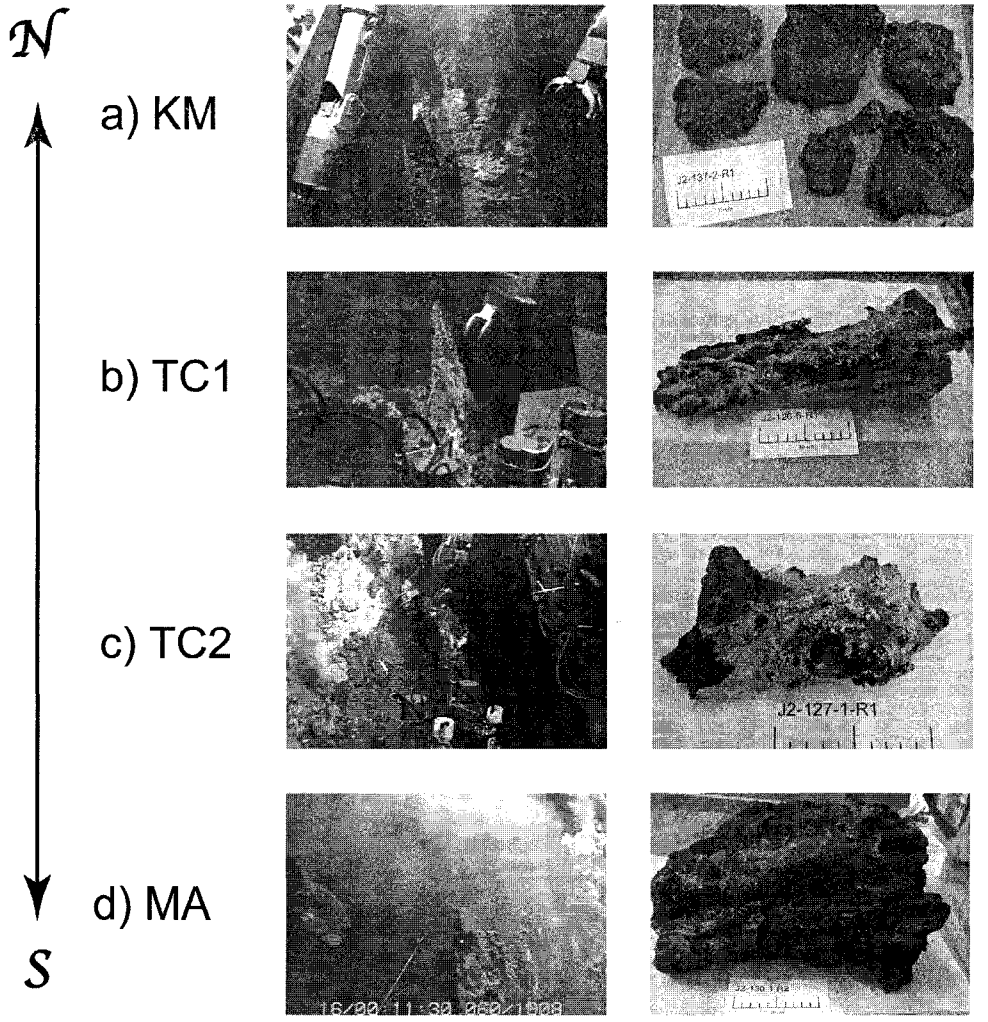

Figure 12. Chimney samples collected along the Eastern Lau Spreading Center and Valu Fa Ridge. As indicated by the arrow, samples are ordered in regard to the relative geographic location of the vent field from which they originate.

colonized by alvinellid worms. TC2 was a softer deposit mostly composed of wurtzite, but also containing an outer layer of sphalerite, marcasite, and pyrite (Fig. 13). Despite major differences in pH (4.5 for TC1, 3.6 for TC2), the overall

chemistry of fluids emitted by the two chimneys was similar. They both contained low concentrations of gases (H_2S, CH_4) and metals (Fe, Mn) (Table III, Seewald, Wheat, Mottl, pers. comm.).

The chimney sampled at the southernmost site, Mariner (sample MA, Fig. 12d), was a hard deposit with a mineralogically zoned wall. The outer surface of the sample was covered with iron oxides (Fig. 12d), and contained sphalerite, chalcopyrite, barite, and anhydrite (Fig. 13). MA's fluids (pH 2.5) contained low gas concentrations and high concentrations of reduced iron and manganese (Table III, Seewald, Wheat, Mottl, pers. comm.).

Quantification of 16S rRNA genes

Archaeal genes appeared to dominate the pool of 16S rRNA genes associated with all DNA extracts (Table IV). On the basis of general trends in numbers of 16S rRNA gene copies in archaeal and bacterial genomes (Acinas et al., 2004), these results suggest that archaeal cells were more abundant than bacterial cells on the surface of the chimneys sampled in this study. However, gene copy numbers presented in this study should not be considered as absolute. In particular, proportions of archaeal 16S rRNA genes might be overestimated due to the unspecific amplification of bacterial genes with primers designed for Archaea. Nevertheless, several lines of evidence demonstrate that archaeal genes were successfully amplified from all samples during these Q-PCR analyses (see Results section of Chapter 3).

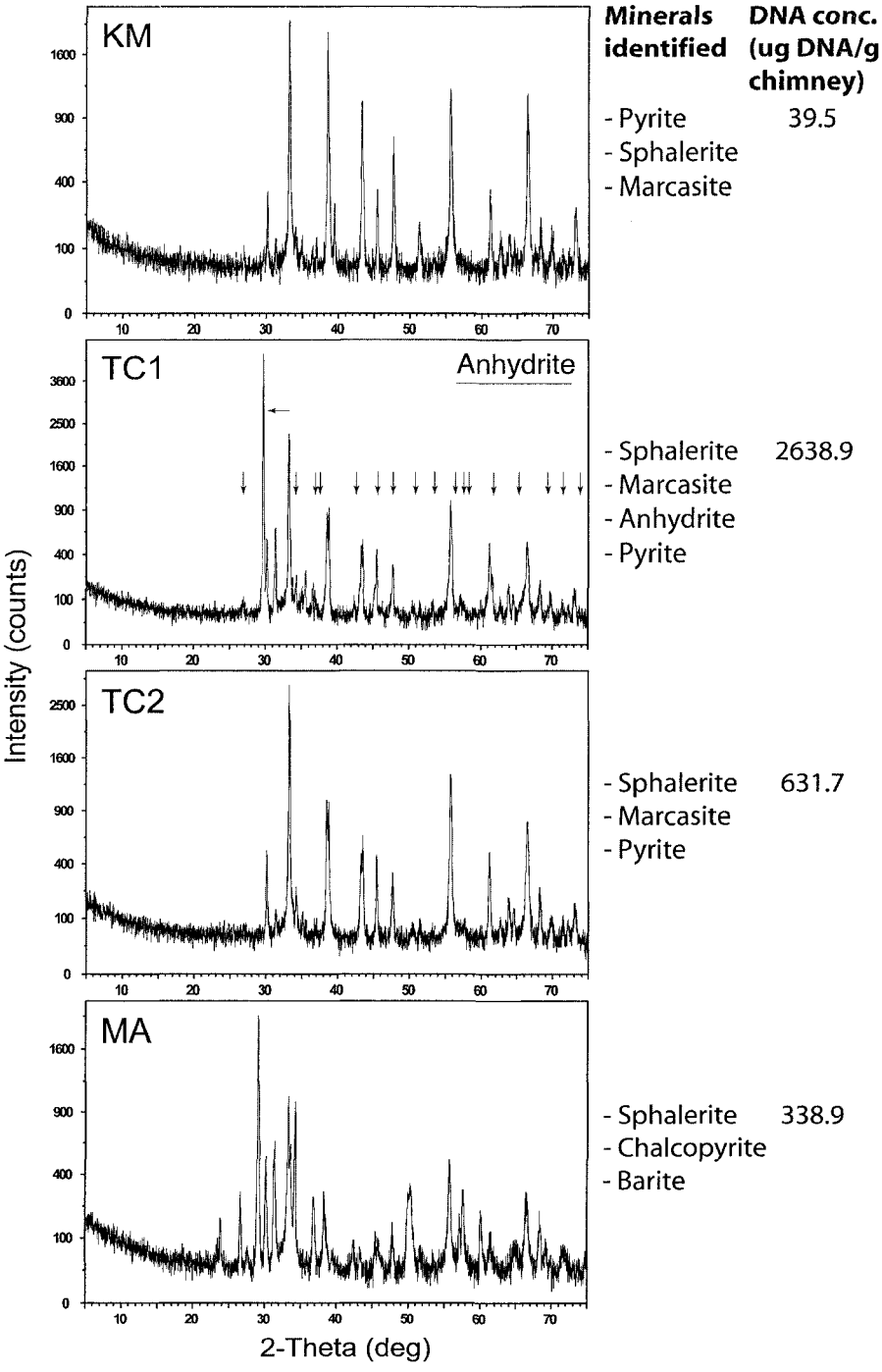

Figure 13. Mineral composition and DNA content of chimney samples collected on the Eastern Lau Spreading Center and Valu Fa Ridge. Minerals are identified by their patterns of X-ray diffraction based on JCPDS cards.

Table III. Characteristics of end-member fluids associated with chimneys collected at the Eastern Lau Spreading Center and Valu Fa Ridge (Seewald, Wheat, Mottl, pers. comm.).

Vent Field	Temp (°C)	pH (25°C)	H_2S (mmol/L)	H_2 (μmol/L)	CH_4 (μmol/L)	Mn (mmol/kg)	Fe (mmol/kg)
KM	303	3.6	5.58	445	31.0	0.6	2.8
TC1	320	4.5	4.5	177	44.8	0.4	0.2
TC2	302	3.6	4.62	108	47.7	0.3	0.3
MA	323	2.5	6.30	54	5.4	5.7	10.8

Table IV. Quantification of 16S rRNA genes in DNA extracts obtained from four chimneys of the Eastern Lau Spreading Center and Valu Fa Ridge.

Sample	Bacterial 16S rRNA genes (16S rRNA genes/g wet of chimney)[a]	Estimated bacterial OTU richness (95% confidence intervals)	Archaeal 16S rRNA genes (16S rRNA genes/g wet of chimney)[a]	Estimated archaeal OTU richness (95% confidence intervals)
KM	$1.28X10^9 \pm 4.39X10^8$ (1.68%)	81 (45-193)	$7.47X10^{10} \pm 1.01X10^{10}$ (98.32%)	6 (5-19)
TC1	$2.23X10^{10} \pm 9.21X10^9$ (0.54%)	26 (19-55)	$4.13X10^{12} \pm 1.20X10^{11}$ (99.46%)	5 (4-17)
TC2	$3.62X10^9 \pm 1.62X10^9$ (4.28%)	33 (22-82)	$8.09X10^{10} \pm 7.09X10^9$ (95.72%)	10 (8-23)
MA	$8.80X10^{10} \pm 2.73X10^{10}$ (19.3%)	100 (54-240)	$3.67X10^{11} \pm 4.92X10^{10}$ (80.7%)	20 (13-55)

[a]In parentheses, proportion of total 16S rRNA genes detected (bacterial and archaeal). Note that concentrations of archaeal genes might be overestimated due to potential unspecific PCR amplification.

Comparison of microbial community structures

As indicated by estimates of OTU richness calculated with DOTUR (97% similarity definition for OTU), 16S rRNA gene diversity was low in all archaeal clone libraries (Table IV). Consequently, rarefaction analyses indicated that our sampling effort was adequate to obtain an accurate representation of archaeal diversity in all samples (data not shown). In contrast, estimates of bacterial OTU richness ranged from 26 in TC1 (19-55 95% confidence interval) to 100 in MA (54-240 95% confidence interval). Thus, the sequencing of 50 clones per bacterial clone library only provided a representation of the numerically dominant populations in each community.

Treeclimber analyses and estimates of shared OTUs indicated that archaeal and bacterial communities were distinct in each chimney sample. Archaeal ($p = 0.006$) and bacterial ($p = 0.008$) 16S rRNA genes were not randomly distributed between clone libraries. Furthermore, overlaps in OTU composition between communities were rare. In Archaea, only TC2 and MA shared some OTUs (estimated 2.6% of the total combined OTUs in the two samples). Although all bacterial clone libraries shared some OTUs, the shared percentages were below 12% except for TC2 and MA (32.3%). The most dissimilar bacterial libraries were TC1 and MA (4.2%).

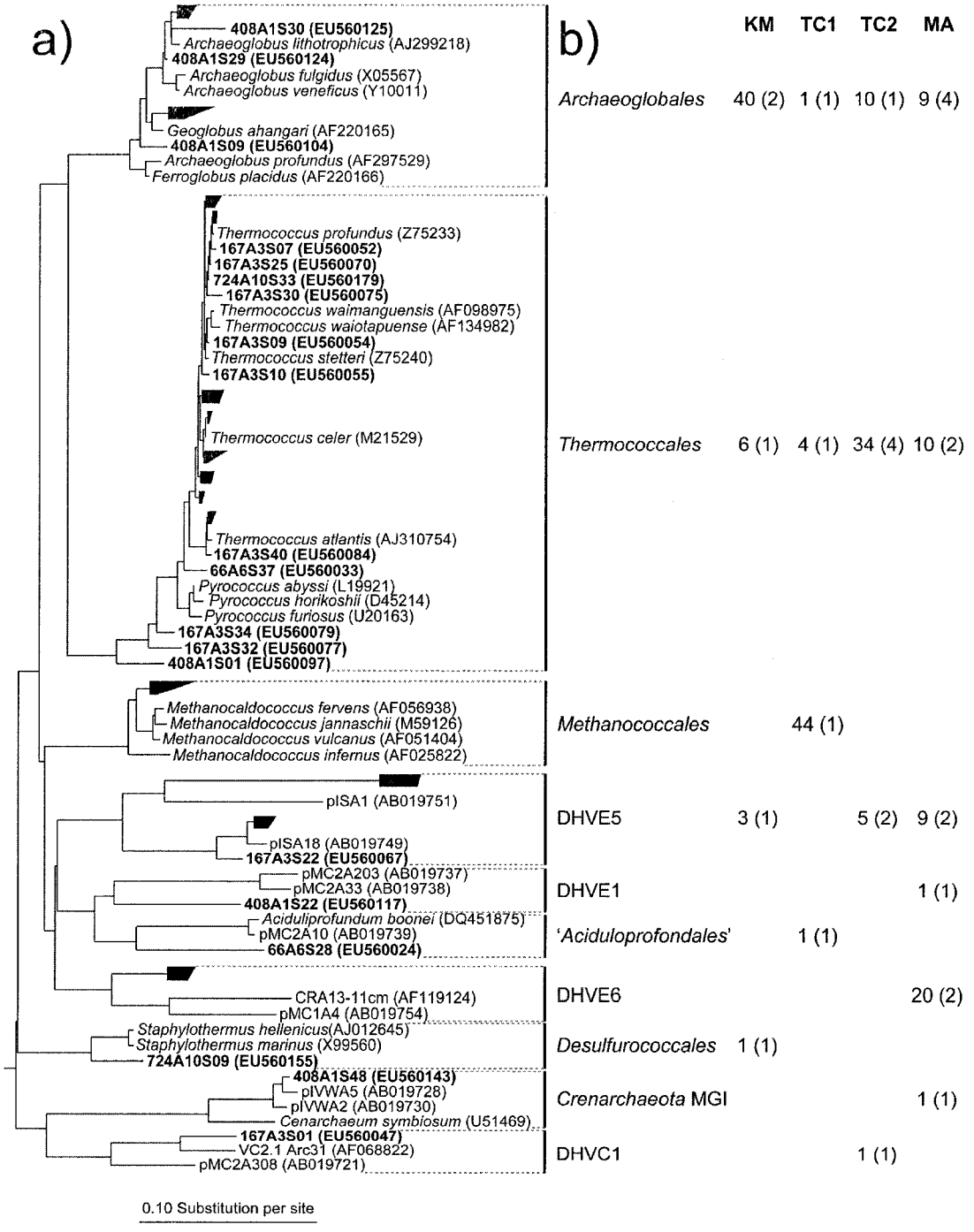

Figure 14. a) Phylogenetic relationship of archaeal 16S rRNA genes as determined by a neighbor-joining analysis. Wedges represent clades formed of sequences collected in this study, and their horizontal dimensions reflect the degree of divergence within the group.

Outgroups were *Aquifex pyrophilus* (M83548) and *Thermotoga maritima* (M21774). b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries. In parentheses, the number of OTUs (97% similarity) observed in each order-level lineage.

Microbial diversity

Phylogenetic analyses of the 16S rRNA gene sequences confirmed that each chimney harbored specific archaeal and bacterial communities. For example, the archaeal clone library from TC1 was largely dominated by representatives of the genus *Methanocaldococcus* (44/50 phylotypes), whereas no other sample contained representatives of this lineage (Fig. 14). Similarly, phylotypes belonging to order-level lineages *Thermococcales*, *Archaeoglobales*, and DHVE6 were dominant in TC2, KM, and MA respectively, but were not observed in large numbers in any other clone library. Nevertheless, members of several orders were detected in multiple communities. This was the case of *Archaeoglobales* and *Thermococcales*, which were present in all samples. Despite the fact that it only constituted a minor component of the communities, the clade of uncultivated Archaea DHVE5 was detected in TC2, KM, and MA.

Although *Epsilonproteobacteria* were detected in all libraries, only a few lineages were present in more than one chimney. These lineages included the Epsilon Group F, D, B, *Gammaproteobacteria*, and *Cytophaga-Flexibacter-Bacteroides*. Nevertheless, each chimney contained a different distribution of

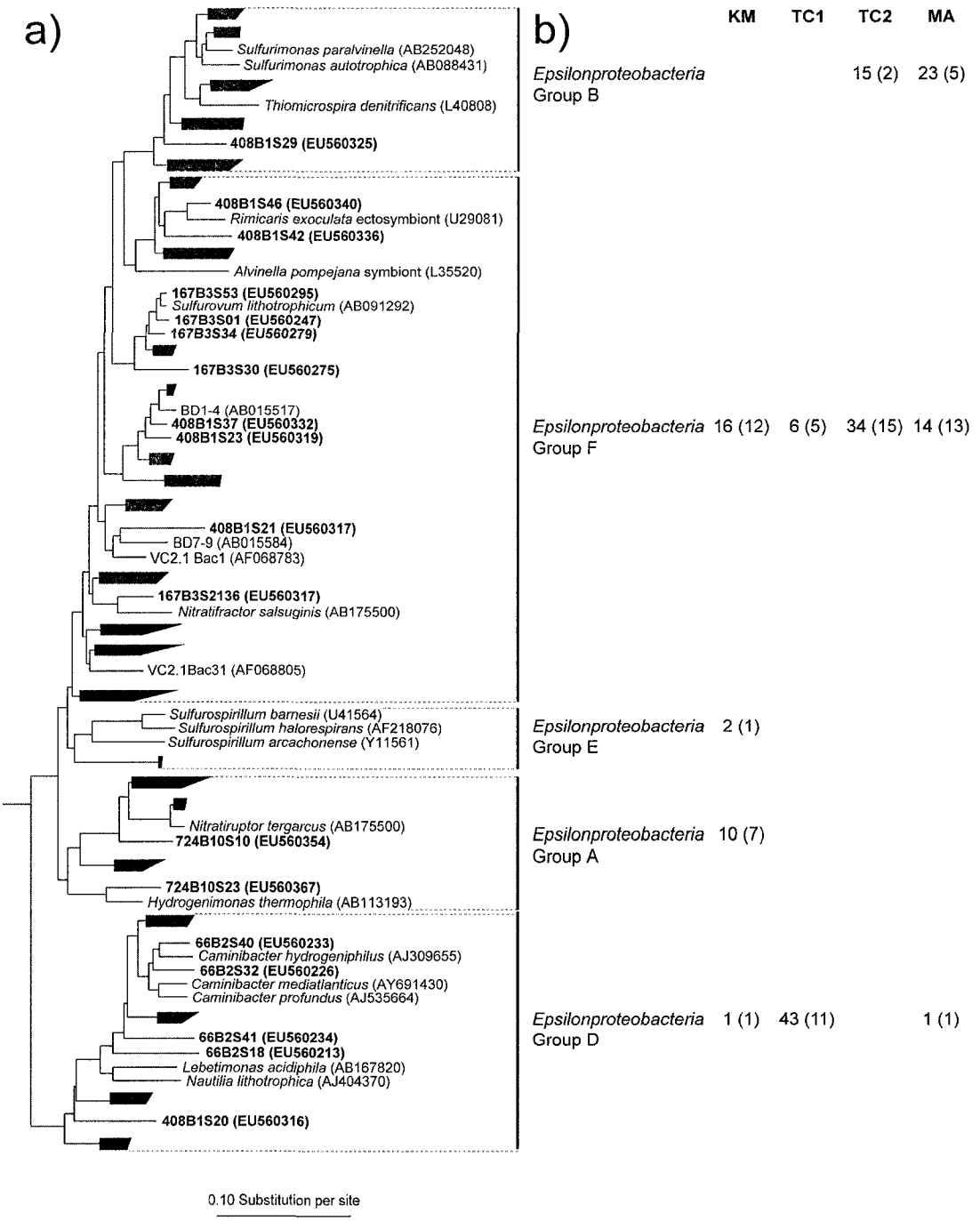

Figure 15. a) Phylogenetic relationship of epsilonproteobacterial 16S rRNA genes as determined by a neighbor-joining analysis. Wedges represent clades formed of sequences collected in this study, and their horizontal dimensions reflect the degree of divergence

within the group. Outgroups were *Methanocaldococcus jannaschii* (M59126) and *Thermococcus celer* (M21529). b) A summary of phylogenetic divisions identified in this study, with the number of 16S rRNA gene sequences obtained from each division in the different clone libraries. In parentheses, the number of OTUs (95% similarity) observed in each order-level lineage.

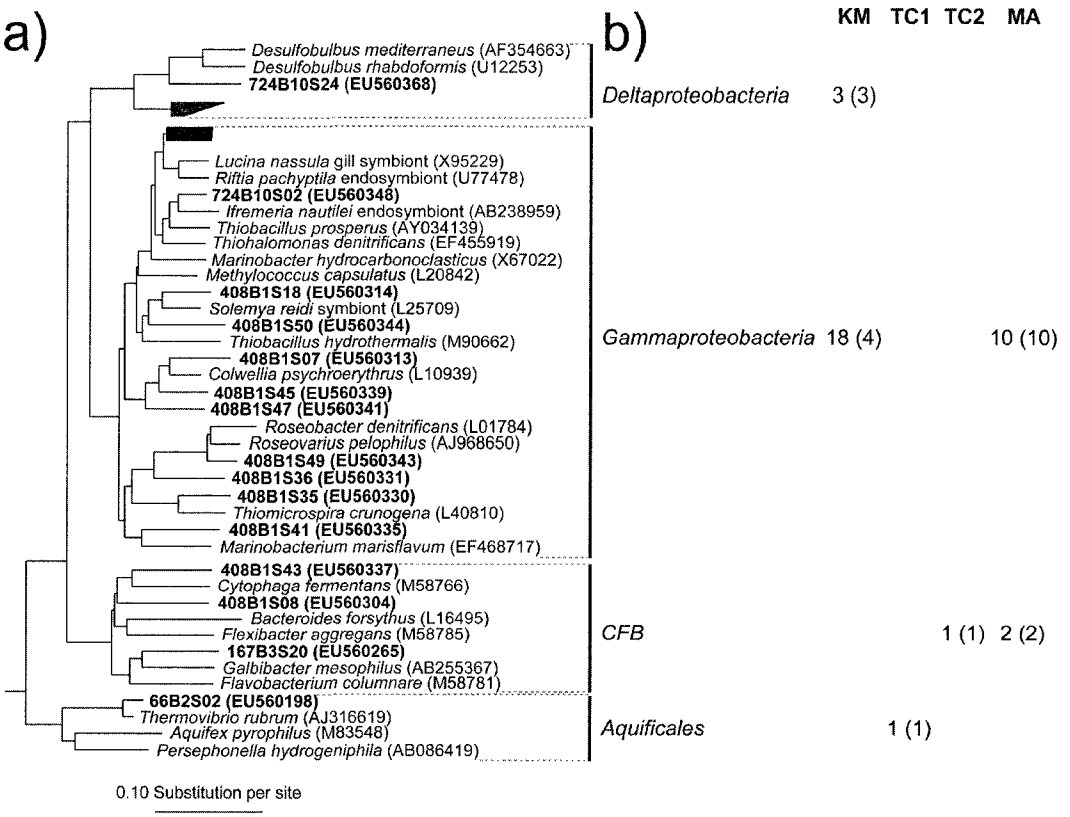

Figure 16. a) Phylogenetic relationship of bacterial 16S rRNA genes as determined by a neighbor-joining analysis. Wedges represent clades formed of sequences collected in this study, and their horizontal dimensions reflect the degree of divergence within the group. Outgroups were *Methanocaldococcus jannaschii* (M59126) and *Thermococcus celer* (M21529). b) A summary of phylogenetic divisions identified in this study, with the

number of 16S rRNA gene sequences obtained from each division in the different clone libraries. In parentheses, the number of OTUs (97% similarity) observed in each order-level lineage.

bacterial phylotypes (Fig. 15, 16). TC1 was the only sample that contained a large number of Epsilon Group D representatives (43/50 phylotypes). Despite the prevalence of Epsilon Group B and F in both TC2 and MA, MA contained several *Gammaproteobacteria* phylotypes (10/50), which were not present in TC2. Despite the presence of numerous *Gammaproteobacteria* phylotypes (18/50) and Epsilon Group F phylotypes (16/50) in KM, the sample was the only one containing phylotypes belonging to Epsilon Group A, E, and *Deltaproteobacteria.*

4.5. Discussion

Microbial communities associated with white spires of Tow Cam vent field

To compare microbial communities associated with mature chimneys formed in the same ELSC vent field, we collected two white spires from Tow Cam (TC1 and TC2). Located in the northern section of the ELSC, Tow Cam vent field is a basalt-hosted hydrothermal system emitting fluids with mid-ocean ridge characteristics (Seewald et al., 2005). Analyses of community composition revealed a very limited overlap in microbial diversity between the two communities. Although variability in fluid chemistry can sometimes explain differences in microbial diversity associated with vents from the same field

(Nakagawa et al., 2005, Perner et al., 2007), the similarities between fluids emitted from TC1 and TC2 suggests that it was not the case in our samples. On the other hand, some differences were observed between the two chimneys, including the presence of *Alvinellids* on TC1, the overall hardness of this chimney compared to TC2, and the presence of anhydrite on the surface of TC1.

The presence of anhydrite is significant, since it suggests that temperature was higher on the surface of TC1 than on TC2 at the time of sampling. Anhydrite possesses a reverse solubility, and is only stable at temperatures above ~130°C (Haymon and Kastner, 1981). As indicated by Hannington et al. (1995), the absence of anhydrite from a chimney's frame often results from the dissolution of the mineral following a prolonged exposure to temperatures below its precipitation threshold. Thus, a difference in temperature might explain the disparity in microbial diversity observed between TC1 and TC2. The presence of abundant 16S rRNA genes belonging to the *Epsilonproteobacteria* group D in TC1, and *Epsilonproteobacteria* group F and B in TC2 support this interpretation. Cultured representatives of the *Epsilonproteobacteria* group D include species belonging to the genera *Caminibacter*, *Nautilia*, and *Lebetimonas* (Alain et al., 2002b; Mirochnishenko et al., 2002; Takai et al., 2005a), which are all strictly anaerobic sulfur-reducing thermophiles. In contrast, *Epsilonproteobacteria* groups F and B are represented in culture collections by microaerophilic sulfide- and/or sulfur-oxidizing mesophiles (species of the genus *Sulfurovum* for group F, and *Sulfurimonas* for group B; Inagaki et al., 2003; 2004).

The temperature differences that may have altered microbial community composition in TC1 and TC2 may have been caused by variability in seawater-fluid mixing style between the two deposits. In TC1, an outward advection of hydrothermal fluid would have increased *in situ* temperature on the chimney's outer surfaces, allowed anhydrite precipitation, and created mostly anoxic conditions inside the chimney's walls (Tivey, 2004). Although modeling of *in situ* conditions has yet to be conducted for white spires, the presence of networks of interconnected channels within the sphalerite-dominated matrices (Koski et al., 1994) suggest that fluids could easily percolate through their walls if they are emitted at high velocities. In addition to the presence of the *Epsilonproteobacteria* Group D, the detection of a lineage of putative anaerobic hyperthermophiles (*Methanocaldococcus* spp.) further strengthens this interpretation. Thermodynamic models predict their occurrence (McCollom and Shock, 1997; Tivey, 2004), but methanogens and sulfur-reducers should not dominate microbial communities colonizing active chimneys unless anaerobic conditions prevail throughout their walls. To our knowledge, the only other high temperature chimney dominated by putative hyperthermophilic methanogens was collected at Guaymas Basin (BM4, Pagé et al., 2008). The latter structure was a young (4-day old) and porous deposit characterized by a steep thermal gradient (2°C to 110°C within the first cm of the chimney's surface) that was also likely created by outward advection of hydrothermal fluids. Although the isotopic composition of sulfur in anhydrite grains from black smokers (Spiess et al., 1980; Shanks et al.,

1981) indicates that seawater sulfate must have been present inside the wall of TC1, models of *in situ* conditions demonstrate that this mixing style could have kept sulfate in low concentrations (Tivey, 2004) that would allow anhydrite precipitation, but not select strictly for sulfate-reducing microorganisms.

The presence of abundant populations of putative mesophilic sulfide- and sulfur –oxidizers (*Epsilonproteobacteria* Group F,B) suggest that oxic microhabitats were available at low temperatures (<40˚C) in TC2. Although these conditions are predicted to exist in chimneys characterized by different mixing styles, they are most likely to occur inside the walls of deposits when fluids mix by diffusion of inward advection of seawater (Tivey, 2004). With the addition of heterotrophs (*Thermococcales*), the community associated with TC2 is reminiscent of microbial communities observed in white spires (Hoek et al., 2003; Nakagawa et al., 2005; Kormas et al., 2006) and black smokers (Schrenk et al., 2003; Pagé et al., 2004; Nakagawa et al., 2005) collected at vent fields of various mid-ocean ridges.

Microbial communities associated with black smokers of Kilo Moana and Mariner vent fields

To compare microbial communities associated with mature chimneys formed at different ELSC vent fields, we also collected sulfide deposits from Kilo Moana (KM) and Mariner (MA). Despite considerable differences in end-member (undiluted) fluid concentrations of H_2, CH_4, Mn and Fe, the microbial

communities associated with KM and MA shared several lineages with the community harbored by TC2 (*Epsilonproteobacteria* group F, *Archaeoglobales*, *Thermococcales*, DHVE5). In light of the high amounts of energy available from the aerobic oxidation of sulfur compounds (McCollom and Shock, 1997; Tivey, 2004), the presence of the *Epsilonproteobacteria* group F in all these samples is not surprising. This lineage of mesophilic sulfide- and sulfur-compound oxidizers is widespread at mid-ocean ridges (Takai et al., 2006b), and is often detected on the outer surface of active chimneys (Hoek et al., 2003; Pagé et al., 2004; Nakagawa et al., 2005; Kormas et al., 2006). Although factors controlling their distribution are not as clear, members of orders *Archaeoglobales* and *Thermococcales* are also common in sulfide deposits (Takai et al., 2006b).

Nevertheless, some features of microbial communities that were specific to KM or MA might be linked to particular hydrothermal fluid compositions. For example, the presence of mesophilic sulfate-reducers (*Deltaproteobacteria*) in KM suggests that the oxic/anoxic transition occurred at a lower temperature than in TC2. This interpretation is in accordance with the high concentration of H_2 measured in fluids discharged from this chimney. As indicated by Tivey (2004), H_2 concentration is one of the major factors determining the temperature of this redox boundary in active chimneys. Based on the presence of *Epsilonproteobacteria* group E phylotypes in KM, the presence of anaerobic conditions at low temperatures might also be linked to the consumption of O_2 by microbial populations conducting aerobic respiration. This division is represented

by the genus *Sulfurospirillum*, which contains several species capable of respiration under microaerophilic conditions (Stolz et al., 2001).

MA contained several lineages that were not detected in any other ELSC chimney analyzed in this study (DHVE6, DHVE1, *Crenarchaeota* Marine Group I, *Geoglobus* spp.). Most of these lineages contain no or only a few cultured species, which considerably limits any interpretation of their physiological characteristics. However, the presence of the putative iron-reducing *Geoglobus* spp. is intriguing, as it not a common lineage in 16S rRNA gene surveys. Although iron contained in hydrothermal fluids (FeII) is not readily available to these microorganisms, fluids emitted from MA contained a significantly higher concentration of iron then fluids discharged by other TC1, TC2, and KM. In addition to a prohibitive effect on macrofaunal colonization, the composition of fluids from this vent field might favor an enhanced iron cycle in active sulfide deposits.

4.6. Conclusion

This study presents preliminary assessments of microbial diversity in active deposits along the Eastern Lau Spreading Center and Valu Fa Ridge. Previous observations have suggested that microbial communities associated with basalt- and andesite-hosted systems are different (Reysenbach, pers. comm.). Following the site-specific characteristics of lavas and hydrothermal fluids, the geochemistry of chimney samples collected in our study was related to the vent fields where

they were collected. However, each deposit harbored a distinct microbial community, even though two of the chimneys were formed by similar fluids and shared multiple morphological and mineralogical characteristics. These results demonstrate how little is known about the environmental and biological factors shaping microbial communities at deep-sea hydrothermal vents. Nevertheless, some patterns of community composition identified in our study could potentially be attributed to specific fluid mixing styles and chemistries. Although the effects of chemistry variability were not as striking as in ultramafic-hosted systems (e.g. Schrenk et al., 2004; Perner et al., 2007), microbial lineages specifically associated with chimneys from Kilo Moana or Mariner appeared linked to high H_2 or Fe fluid concentrations. In addition, differences in community composition between chimneys sharing mineralogical, morphological, and hydrothermal fluid characteristics appeared to support models indicating that differences in fluid mixing styles can have profound effects on temperature and chemistry within sulfide deposits (Tivey, 2004). Combined with similar studies conducted at other vent sites, these patterns are starting to reveal the quasi ubiquitous distribution of some lineages (*Epsilonproteobacteria* group F, *Archaeoglobales*, *Thermococcales*) among hydrothermal vent fields.

CHAPTER 5: Conclusion

To date, our knowledge of microbial diversity associated with high temperature deep-sea vent chimneys is limited to data generated by a handful of studies (Takai et al., 2006b). Combined with the characterization of new microorganisms isolated from various vent fields (see reviews by Jannasch, 1995; Karl, 1995; Reysenbach et al., 2002; Mirochnishenko and Bonch-Osmolovskaya, 2006), these experiments have provided valuable information regarding the nature and range of microhabitats available within active deposits. Nevertheless, the environmental and biological constraints on resident microbial communities remain poorly defined. The studies presented in this dissertation aimed to improve our understanding of factors controlling microbial community composition and structure by using an integrative approach. As much as possible, microbiological data were placed in their environmental context through analyses of various parameters defining *in situ* conditions.

Integrative approaches have been limited by technological hurdles associated with the measurement of conditions within actively precipitating mineral deposits characterized by extreme physical and chemical gradients. However, this challenge was partly overcome by the design of a new generation of instruments with improved analytical capacities. In Chapter 2, thermocouple arrays were used to obtain *in situ* temperature measurement across the walls of three active deposits from the Guaymas Basin hydrothermal vent field. By comparing the thermal gradients associated with each chimney to the distribution of microorganisms, it

was possible to demonstrate that *in situ* temperature put important constraints on the depth of microbial colonization within these deposits. Nevertheless, data suggesting that some microorganisms can withstand supraoptimal temperatures were obtained from this study (Pagé et al., 2008). Although this capacity had been previously demonstrated in laboratory settings (Trent et al., 1990; 1994; Holden and Baross, 1993), environmental evidence was still elusive. Indications recently provided by mineralogical data had also narrowed the range of temperature expected to allow the development of stable microbial habitats (Kormas et al., 2006), but our study provided the first combination of microbiological and temperature data directly collected in situ. In addition, successive deployments or arrays over fluids from a singe vents allowed us to demonstrate that microbial communities in 4- and 72-day-old chimneys were not constituted of the same lineages (e.g. *Methanocaldococcus* spp. vs. a diversity of mostly heterotrophic lineages).

In Chapter 3, the composition and structure of microbial communities associated with newly formed (stage I) and mature chimneys (stage II) were described. This study built on previous reports (Reysenbach et al., 2002; 2002; Pagé et al., 2008), which suggested that microbial communities undergo succession at deep-sea hydrothermal vents. Although 16S rRNA gene diversity assessments had been conducted separately on chimneys from each of the two stages (stage I: McCliment et al., 2006, stage II: Takai and Horikoshi, 1999; Takai et al., 2001; Schrenk et al., 2003; Hoek et al., 2003; Pagé et al., 2004; Nakagawa

et al., 2005; Kormas et al., 2006), data presented in our study constituted the first report of microbial diversity associated with the two types of chimneys collected from the same vent field. Furthermore, descriptions and comparisons of community structure had never been conducted for deep-sea vent microbial communities. In addition to evidence suggesting that microbial communities diversify over time, our data further demonstrated that the pioneer community in a Juan de Fuca chimney was constituted of a very limited number of hyperthermophilic and autotrophic archaeal populations (closely related to *Archaeoglobus lithotrophicus*), whereas multiple lineages of heterotrophic Archaea and Bacteria were present in mature deposits (e.g. *Methanosarcinales*, '*Aciduliprofundales*', *Thermococcales*). These results suggest that internal cycling of organic compounds and temporal evolution of chimney walls have important effects on microbial succession in active deposits. Thus, our study provided new insights into the mechanisms underlying microbial succession at the high temperature end of deep-sea hydrothermal vent ecosystems.

In Chapter 4, the composition and structure of microbial communities associated with four mature chimneys of Lau Basin hydrothermal vents were described (KM = Kilo Moana, TC1 and TC2 = Tow Cam, MA = Mariner). These experiments follow the recent discovery of new hydrothermal vent fields along the Eastern Lau Spreading Center (ELSC) and Valu Fa Ridge (VFR) (Michael and Seewald, 2007). The ELSC and VFR are among the most geologically complex axes on Earth, as crustal lithology and magma composition are gradually

influenced by the subducted Pacific Plate (Pearce and Stern, 2006). As a result, important chemical gradients are observed in the composition of hydrothermal fluids emitted between Kilo Moana (northernmost vent field) and Vai Lili (southernmost vent field) (Seewald et al., 2005). Although variability in fluid composition (concentration of H_2 and Fe) appeared to have an impact on minor components of microbial communities, data obtained in this study suggested that differences in fluid mixing styles could also profoundly alter the composition of microbial communities. Although these results were predicted by models of *in situ* conditions within active sulfide deposits (Tivey, 2004), environmental evidence had never been obtained.

Integrative studies combining descriptions of microbial communities and assessments of environmental conditions have considerably improved our understanding of the interactions between microorganisms and their environment. The discovery of new hydrothermal systems and the availability of new analytical tools provide exciting perspectives for microbial ecologists using this approach in the field of deep-sea hydrothermal vent microbiology. However, integrative studies will have to be replicated in order to allow a higher resolution comprehension of the links between large-scale geological processes and microbial life at deep-sea hydrothermal vents. Due to time constraints associated with the generation and analysis of 16S rRNA gene sequence data, current studies usually focus on small numbers of samples (e.g. n = 1 to 3 for high temperature chimneys). As a result, our understanding of deep-sea vent microbial ecology is

based on limited observations and covers small spatial and temporal scales. For example, the work presented in Chapter 3 of this dissertation presents the microbial diversity associated with a few locations (4-6 sampling points) within only three 4- or 72-day-old deposits. Thus, the meanings of spatial and temporal trends in microbial community composition indicated by these results have to be interpreted carefully.

Although the study of microbial communities over larger spatial and temporal scales will remain limited by the remote nature of deep-sea hydrothermal vents, the advent of new nucleotide sequencing technologies (e.g. pyrosequencing, Margulies et al., 2005) should alleviate some of the limitations inherent to the production of 16S rRNA gene sequences, and thus allow assessments of larger sample sets. Studies utilizing these technologies have already provided more detailed descriptions of microbial communities in some vent environments (Sogin et al., 2006; Huber et al., 2007). With these large datasets, the next scientific hurdles will be associated with the analysis and storage of the data (Hugenholtz , 2007).

Microbial ecologists will also have to overcome limitations associated with the approach of microbial community description based on 16S rRNA gene sequencing. This approach has added a formidable tool to culture-based techniques and allowed microbiologists to reveal the uncultured portion of microbial diversity. However, interpretations of microbial community composition and structure based on PCR amplification of 16S rRNA gene are

vulnerable to a series of potential biases (see section 1.3.2.). Furthermore, interpretations of *in situ* microbial functions from phylogenetic relationships to cultured microorganisms are likely misleading in some cases. The solution to these problems may reside in the environmental genomics approach pioneered by the work of Dr. Jo Handelsman, Dr. Edward F. DeLong, Dr. Jillian F. Banfield and their colleagues (e.g. Rondon et al., 2000; Béjà et al., 2001; Tyson et al., 2004). Combined with transcriptomics and proteomics, the sequencing of genome fragments from naturally occurring microbial populations can reveal the real composition and structure of microbial communities, the genetic potential for specific microbial processes, and strong indications on whether these processes occur or not at the time of sampling. One can also predict that increasing nucleotide sequencing and analysis capacities will soon open the door to studies using temporal and spatial metagenomics to monitor the evolution of microbial systems. Nevertheless, studies relying on these new approaches only reach their full potential when the observed microorganisms are amenable to cultivation, and thus can be monitored in the laboratory.

Deep-sea hydrothermal vent environments harbor some of the most remote and least understood ecosystems on Earth. Despite their position at the base of these ecosystems, hydrothermal vent microbial communities remain understudied. The efforts of physicists, chemists, geologists, and biologists have highlighted the potential effects of a series of factors on the composition and structure of microbial communities (Fig. 1). However, the links between most factors and

microbial communities have not yet been demonstrated. The three studies presented in this document were designed to demonstrate the effects of: (1) temperature gradients, (2) products of biological activity, (3) mineral composition of chimneys, and (4) chemistry and pH of hydrothermal fluids. Although trends were demonstrated, the significance of these interactions on global processes in hydrothermal ecosystems remains to be demonstrated.

REFERENCES

Acinas, S.G., Marcelino, L.A., Klepac-Ceraj, V., and Polz, M.F. (2004) Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. *Journal of Bacteriology* **186:**2629-2635.

Acinas, S.G., Sarma-Rupavtarm, R., Klepac-Ceraj, V., and Polz, M.F. (2005) PCR-induced sequence artifacts and bias: insights from comparison of two 16S rRNA clone libraries constructed from the same sample. *Applied and Environmental Microbiology* **71:**8966-8969.

Alain, K., Olagnon, M., Desbruyères, D., Pagé, A., Barbier, G., Juniper, S.K., Quérellou, J., and Cambon-Bonavita, M.A. (2002a) Phylogenetic characterization of the bacterial assemblage associated with mucous secretions of the hydrothermal vent polychaete *Paralvinella palmiformis*. *FEMS Microbiology Ecology* **42**:463-476.

Alain, K., Querellou, J., Lesongeur, F., Pignet, P., Crassous, P., Raguénès, G., Cueff, V., and Cambon-Bonavita M.A. (2002b) *Caminibacter hydrogeniphilus* gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium isolated from an East Pacific Rise hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* **52**:1317-1323.

Alain, K., Zbinden, M., Le Bris, N., Lesongeur, F., Quérellou, J., Gaill, F., and Cambon-Bonavita, M.A. (2004) Early steps in microbial colonization processes at deep-sea hydrothermal vents. *Environmental Microbiology* **6**:227-241.

Allers, E., Gómez-Consarnau, L., Pinhassi, J., Gasol, J.M., Simek, K., and Pernthaler, J. (2007) Response of *Alteromonadaceae* and *Rhodobacteriaceae* to glucose and phosphorus manipulation in marine mesocosms. *Environmental Microbiology* **9**:2417-2429.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215**:403–410.

Amend, J.P., and Shock, E.L. (1998) Energetics of amino acid synthesis in hydrothermal ecosystems. *Science* **281**:1659-1662.

Antoine, E., Cilia, V., Meunier, J. R., Guezennec, J., Lesongeur, F., and Barbier, G. (1997) *Thermosipho melanesiensis* sp. nov., a new thermophilic anaerobic bacterium belonging to the order *Thermotogales*, isolated from deep-sea hydrothermal vents in the Southwestern Pacific Ocean. *International Journal of Systematic Bacteriology* **47**:1118-1123.

Ashelford, K.E., Chuzhanova, N.A., Fry, J.C., Jones, A.J., and Weightman, A.J. (2005) At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. *Applied and Environmental Microbiology* **71**:7724-7736.

Bach, W., Humphris, S.E., and Fisher, A.T. (2004) Fluid flow and fluid-rock interaction within ocean crust: reconciling geochemical, geological, and geophysical observations. *In* The subseafloor biosphere at mid-ocean ridges. W.S.D. Wilcock, E. F. DeLong, D.S. Kelley, J.A. Baross, and S.C. Cary (eds) Geophysical monograph series. Washington, DC. p. 99-118.

Ballard, R.D. (1977) Notes on a major oceanographic find. *Oceanus* **20**:35-44.

Bano, N., Ruffin, S., Ransom, B., and Hollibaugh, J.T. (2004) Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. *Applied and Environmental Microbiology* **70**:781-789.

Baross, J.A., Lilley, M.D., and Gordon, L.I. (1983) Is the CH_4, H_2 and CO venting from submarine hydrothermal systems produced by thermophilic bacteria? *Nature* **298**:366-368.

Baross (1998) Do the geological and geochemical records of the early Earth support the prediction from global phylogenetic models of a thermophilic cenancestor. *In* Thermophiles: a key to molecular evolution and the origin of life? J. Wiegel and M.W.W. Adams (eds). Taylor & Francis. Philadelphia, PA. p. 1-18.

Baross and Deming (1995) Growth at high temperatures: isolation and taxonomy, physiology, and ecology. *In* The microbiology of deep-sea hydrothermal vents. Karl, D.M. (ed). CRC Press. Boca Raton, FL. p. 169-217.

Bazylinski, D.A., Farrington, J.W., and Jannasch, H.W. (1988) Hydrocarbons in surface sediments from a Guaymas Basin hydrothermal vent site. *Organic Geochemistry* **12**:547-558.

Béjà, O., Spudich, E.N., Spudich, J.L., Leclerc, M., and DeLong, E.F. (2001) Proteorhodopsin phototrophy in the ocean. *Nature* **411**:786-789.

Boucher, Y., Huber, H., L'Haridon, S., Stetter, K.O., and Doolittle, W.F. (2001) Bacterial origin for the isoprenoid biosynthesis enzyme HMG-CoA reductase

of the archaeal orders *Thermoplasmatales* and *Archaeoglobales*. *Molecular and Biological Evolution* **18:**1378-1388.

Bowers, T.S., Von Damm, K.L., and Edmond, J.M. (1985) Chemical evolution of mid-ocean ridge hot springs. *Geochimica et Cosmochimica Acta* **49**:2239-2252.

Brazelton, W.J., Schrenk, M.O., Kelley, D.S., and Baross, J.A. (2006) Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. *Applied and Environmental Microbiology* **72**:6257-6270.

Breitbart, M., Wegley, L., Leeds, S., Schoenfeld, T., and Rohwer, F. (2004) Phage community dynamics in hot springs. *Applied and Environmental Microbiology* **70**:1633-1640.

Butterfield, D.A., Jonasson, I.R., Massoth, G.J., Feely, R.A., Roe, K.K., Embley, R.E., Holden, J.F., McDuff, R.E., Lilley, M.D., and Delaney, J.R. (1997) Seafloor eruptions and evolution of hydrothermal fluid chemistry. *Philosophical Transactions of the Royal Society of London* **355**:369–386.

Campbell, B.J., Stein, J.L., and Cary, S.C. (2003) Evidence of chemolithoautotrophy in the bacterial community associated with *Alvinella pompejana*, a hydrothermal vent polychaete. *Applied and Environmental Microbiology* **69**:5070-5078.

Cary, S.C., Cottrell, M.T., Stein, J.L., Camacho, F., and Desbruyères, D. (1997) Molecular identification and localization of a filamentous symbiotic bacteria

associated with the hydrothermal vent annelid, *Alvinella pompejana*. *Applied and Environmental Microbiology* **63**:1124-1130.

Castillo, M., Martín-Orúe, S.M., Manzanilla, E.G., Badiola, I., Martín, M., and Gasa, J. (2006) Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR. *Veterinary Microbiology* **16**:165-170.

Chao, A. (1984) Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* **11**:265-270.

Chevaldonné, P., and Godfroy, A. (1997) Enumeration of microorganisms from deep-sea hydrothermal chimney samples. *FEMS Microbiology Letters* **146**:211-216.

Chyba, C.F., and Phillips, C.B. (2002) Europa as an abode of life. *Origins of Life and Evolution of the Biosphere* **32**:47-68.

Corliss, J.B., Dymond, J., Gordon, L.I., Edmond, J.M., von Herzen, R.P., Ballard, R.D., Green, K., Williams, D., Bainbridge, A., Crane, K., and van Andel, T.H. (1979) Submarine thermal springs on the Galapagos Rift. *Science* **203**:1073-1083.

Corre, E., Reysenbach, A. L., and Prieur, D. (2001) Epsilon-proteobacterial diversity from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. *FEMS Microbiology Letters* **205**:329-335.

Delaney, J.R., Kelley, D.S., Lilley, M.D., Butterfield, D.A., Baross, J.A., Wilcock, W.S., Embley, R.W., and Summit, M. (1998) The quantum event of

oceanic crustal accretion: impacts of diking at mid-ocean ridges. *Science* **281**:222-230.

de la Torre, J.R., Walker, C.B., Ingalls, A.E., Könneke, M., and Stahl, D.A. (2008) Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. *Environmental Microbiology* **10**:810-818.

DeLong, E.F. (1992) Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences of the USA* **89**:5685-5689.

Deming, J.W., and Baross, J.A. (1993) Deep-sea smokers: windows to a subsurface biosphere? *Geochimica et Cosmochimica Acta* **57**:3219-3230.

Dhillon, A., Teske, A., Dillon, J., Stahl, D.A., and Sogin, M.L. (2003) Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. *Applied and Environmental Microbiology* **69**:2765-2772.

Donval, J.P., Charlou, J.L., Douville, E., Knoery, J., Fouquet, Y., Poncevera, E., Baptiste, P.J., and Stievenard German, C. (1997) High H_2 and CH_4 content in hydrothermal fluids from Rainbow site newly sampled at 36°14'N on AMAR segment, Mid-Atlantic Ridge (diving FLORES cruise, July 1997). Comparison with other MAR sites. *EOS* **78**:832.

Edgcomb, V.P., Molyneaux, S.J., Boer, S., Wirsen, C.O., Saito, M., Atkins, M.S., Lloyd, K., and Teske A. (2007) Survival and growth of two heterotrophic hydrothermal vent archaea, *Pyrococcus* strain GB-D and *Thermococcus fumicolans*, under low pH and high sulfide concentrations in combination with high temperature and pressure regimes. *Extremophiles* **11**:329-342.

Eilers, H., Pernthaler, J., and Amann, R. (2000) Succession of pelagic marine bacteria during enrichment: a close look on cultivation-induced shifts. *Applied and Environmental Microbiology* **66**: 4634–4640.

Fouquet, Y.U., Von Stackelberg, J.L., Charlou, J.P., Donval, J., Erzinger, J.P., Foucher, P., Herzig, R., Mühe, S., Soakai, M., Wiedicke, and Whitechurch, H. (1991) Hydrothermal activity and metallogenesis in the Lau back-arc basin. *Nature*, **349**: 778-781.

Fornari, D.J., and Embley, R.W. (1995) Tectonic and volcanic controls on hydrothermal processes at the mid-ocean ridge: an overview based on near-bottom and submersible studies. *In* Seafloor hydrothermal systems: physical, chemical, biological, and geological interactions. S.E. Humphris, R.A. Zierenberg, L.S. Mullineaux, and R.E. Thomson (eds). American Geophysical Union. Washington, DC. p. 1-46.

Fox, G.E., Magrum, L.J., Balch, W.E., Wolfe, R.S., and Woese, C.R. (1977) Classification of methanogenic bacteria by 16S ribosomal RNA characterization. *Proceedings of the National Academy of Sciences of the U.S.A.* **74:**4537-4541.

Früh-Green, G., Connolly, J.A., Plas, A., Kelley, D.S., and Grobéty, B. (2004) Serpentinization of oceanic peridotites: implications for geochemical cycles and biological activity. *In* The subseafloor biosphere at mid-ocean ridges. W.S.D. Wilcock, E. F. DeLong, D.S. Kelley, J.A. Baross, and S.C. Cary (eds) Geophysical monograph series. Washington, DC. p. 119-136.

Geslin, C., Le Romancer, M., Gaillard, M., Erauso, G., and Prieur, D. (2003) Observation of virus-like particles in high temperature enrichment cultures from deep-sea hydrothermal vents. *Research in Microbiology* **154**:303-307.

Giovanonni, S.J., Britschgi, T.B., Moyer, C.L., and Field, K.G. (1990) Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345:**60-63.

Goldfarb, M.S., Converse, D.R., Holland, H.D., and Edmond, J.M. (1983) The genesis of hot spring deposits on the East Pacific Rise, 21°N, *Economic Geology* **5**:184-197.

Haberstroh, P.R., and Karl, D.M. (1989) Dissolved free amino acids in hydrothermal vent habitats of the Guaymas Basin. *Geochimica et Cosmochimica Acta* **53**:2937-2945.

Habicht, K.S., Gade, M., Thamdrup, B., Berg, P., and Canfield, D.E. (2002) Calibration of sulfate levels in the Archean Ocean. *Science* **298**:2372-2374.

Haddad, A., Camacho, F., Durand, P., and Cary, S.C. (1995) Phylogenetic characterization of the epibiotic bacteria associated with the hydrothermal vent polychaete *Alvinella pompejana. Applied and Environmental Microbiology* **61**:1679-1687.

Hannington, M.D, Jonasson, I.R., Herzig, P.M., and Peterson, S. (1995) Physical and chemical processes of seafloor mineralization at mid-ocean ridges. *In* Seafloor hydrothermal systems: physical, chemical, biological, and geological interactions. S.E. Humphris, R.A. Zierenberg, L.S. Mullineaux, and R.E. Thomson (eds). American Geophysical Union. Washington, DC. p. 115-157.

Harmsen, H.J.M., Prieur, D., and Jeanthon, C. (1997a) Distribution of microorganisms in deep-sea hydrothermal vent chimneys investigated by whole-cell hybridization and enrichment culture of thermophilic subpopulations. *Applied and Environmental Microbiology* **63**:2876-2883.

Harmsen, H.J.M., Prieur, D., and Jeanthon, C. (1997b) Group-specific 16S rRNA-targeted oligonucleotide probes to identify thermophilic bacteria in marine hydrothermal vents. *Applied and Environmental Microbiology* **63**:4061-4068.

Haymon, R.M. (1983) Growth history of hydrothermal black smoker chimneys. *Nature* **301**:695-698.

Haymon, R.M., and Kastner, M (1981) Hot spring deposits on the East Pacific Rise at 21°N: preliminary description of mineralogy and genesis. *Earth and Planetary Science Letters* **53:**363-381.

Hedrick, D.B., Pledger, R.D., White, D.C., and Baross, J.A. (1992) *In situ*microbial ecology of hydrothermal vent sediments. *FEMS Microbiology Ecology* **101**:1-10.

Hoek, J., Banta, A.B., Hubler, R.F., and Reysenbach, A.L. (2003) Microbial diversity of a deep-sea hydrothermal vent biofilm from Edmond Vent Field on the Central Indian Ridge. *Geobiology* **2**:119-127.

Holden, J.F., and Baross, J.A. (1993) Enhanced thermotolerance and temperature-induced changes in protein composition in the hyperthermophilic archaeon ES4. *Journal of Bacteriology* **175**:2839-2843.

Holm, N.G. (1992) Why are hydrothermal systems proposed as plausible environments for the origin of life? *Origins of Life and Evolution of the Biosphere* **22**:181-242.

Huber, C., and Wachtershauser, G. (1998) Peptides by activation of amino acids with CO on (Ni,Fe)S surfaces: Implications for the origin of life. *Science* **281**:670-672.

Huber, H., Burggraf, S., Mayer, T., Wyschkony, I., Rachel, R., and Stetter K.O. (2000) *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic Archaea, represented by two new species, *Ignicoccus islandicus* sp nov and *Ignicoccus pacificus* sp nov. *International Journal of Systematic and Evolutionary Microbiology* 50:2093-100.

Huber, H., Hohn, M.J., Rachel, R., Fuchs, T., Wimmer, V.C., and Stetter, K.O. (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* **417**:63-67.

Huber, T., Faulkner, G., and Hugenholtz, P. (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20:**2317-2319.

Huber, J.A., Mark Welch, D.B., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., and Sogin, M.L. (2007) Microbial population structures in the deep marine biosphere. *Science* **318**:97-100.

Hugenholtz, P. (2007) Riding giants. *Environmental Microbiology* **9:**5.

Inagaki, F., Takai, K., Kobayashi, H., Nealson, K.H., and Horikoshi, K. (2003) *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing epsilon-proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* **53**:1801-1805.

Inagaki F, Takai K, Nealson KH, and Horikoshi K. (2004) *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the epsilon-Proteobacteria isolated from Okinawa Trough hydrothermal sediments. *International Journal of Systematic and Evolutionary Microbiology* **54**:1477-1482.

Jackson, C.R., Churchill, P.F., and Roden, E.E. (2001) Successional changes in bacterial assemblage structure during epilithic biofilm development. *Ecology* **82**:555-566.

Jackson, C.R. (2003) Changes in community properties during microbial succession. *Oikos* **101**:444-448.

Jakosky, B.M., and Shock, E.L. (1998) The biological potential of Mars, the early Earth, and Europa. *Journal of Geophysical Research* **103**:19359-19364.

Janecky, D.R., and Seyfried, W.E. (1984) Formation of massive sulfide deposits on oceanic ridge crest: incremental reaction models for mixing between hydrothermal solutions and seawater. *Geochimica et Cosmochimica Acta* **48**:2723-2738.

Jannasch, H.W. (1995) Microbial interactions with hydrothermal fluids. *In* Seafloor hydrothermal systems: physical, chemical, biological, and geological interactions. Humphris, S.E., Zierenberg, R.A., Mullineaux, L.S., and Thomson, R.E. (eds). American Geophysical Union. Washington, DC. p. 273-296.

Jannasch, H.W., and Wirsen C.O. (1979) Chemosynthetic primary production at East Pacific seafloor spreading centers. *Biosciences* **29**:592-598.

Jannasch, H.W., Wirsen, C.O., Nelson, D.C., and Robertson, L.A. (1985) *Thiomicrospira crunogena* sp. nov., a colorless, sulfur-oxidizing bacterium from deep-sea hydrothermal vents. *International Journal of Systematic Bacteriology* **35**:422-424.

Jeanthon, C., L'Haridon, S., Reysenbach, A.L., Corre, E., Vernet, M., Messner, P., Sleytr, U.B., and Prieur, D. (1999) *Methanococcus vulcanius* sp. nov., a novel hyperthermophilic methanogens isolated from East Pacific Rise, and identification of *Methanococcus* sp. DSM4213T as *Methanococcus fervens*. *International Journal of Systematic Bacteriology* **49**:583-589.

Juniper, S. K., Cambon, M.A., Lesongeur, F., and Barbier, G. (2001) Extraction and purification of DNA from organic rich subsurface sediments (ODP Leg 169S). *Marine Geology* **174**:241-247.

Jones, W.J., Stugard, C.E., and Jannasch, H.W. (1989) Comparison of thermophilic methanogens from submarine hydrothermal vents. *Archives of Microbiology* **151**:314-318.

Karl, D.M. (1995) Ecology of free-living, hydrothermal vent microbial communities. In *The microbiology of deep-sea hydrothermal vents*. Karl, D.M. (ed). CRC Press. Boca Raton, FL. p. 35-124.

Karner, M.B., DeLong, E.F., and Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409**:507-510.

Kashefi, K., and Lovley, D.R. (2003) Extending the upper temperature limit for life. *Science* **301**:934.

Kashefi, K., Tor, J.M., Holmes, D.E., Gaw Van Praagh, C.V., Reysenbach, A.L., and Lovley, D.R. (2002) *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. *International Journal of Systematic and Evolutionary Microbiology* **52**:719-728.

Kawka, O.E., and Simoneit, B.R.T. (1987) Survey of hydrothermally-generated petroleums from the Guaymas Basin spreading center. *Organic Geochemistry* **11**:311-328.

Kawka, O.E., and Simoneit, B.R.T. (1990) Polycyclic aromatic hydrocarbons in hydrothermal petroleums from the Guaymas Basin spreading center. *Applied Geochemistry* **5**:17-27.

Kelley, D.S., Karson, J.A., Blackman, D.K., Früh-Green, G.L., Butterfield, D.A., Lilley, M.D., Olson, E.J., Schrenk, M.O., Roe, K.K., Lebon, G.T., Rivizzigno,

P., and AT3-60 Shipboard Party. (2001) An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30 degrees N. *Nature* **412**:145-149.

Kelley, D.S., Karson, J.A., Früh-Green, G.L., Yoerger, D.R., Shank, T.M., Butterfield, D.A., Hayes, J.M., Schrenk, M.O., Olson, E.J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A.S., Brazelton, W.J., Roe, K., Elend, M.J., Delacour, A., Bernasconi, S.M., Lilley, M.D., Baross, J.A., Summons, R.E., and Sylva, S.P. (2005) A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science* **307**:1428-1434.

Kimura, H., Asada, R., Masta, A., and Naganuma, T. (2003) Distribution of microorganisms in the subsurface of the manus basin hydrothermal vent field in Papua New Guinea. *Applied and Environmental Microbiology* **69**:644-648.

Kodama, Y., and Watanabe, K. (2004) *Sulfuricurvum kujiense* gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. *International Journal of Systematic and Evolutionary Microbiology* **54**:2297-2300.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437:543-546.

Kormas, K.A., Tivey, M.K., Von Damm, K., and Teske, A. (2006) Bacterial and archaeal phylotypes associated with distinct mineralogical layers of a white

smoker spire from a deep-sea hydrothermal vent site (9 degrees N, East Pacific Rise). *Environmental Microbiology* **8**:909-920.

Koski, R.A., Jonasson, I.R., Kadko, D.C., Smith, V.K., and Wong, F.L. (1994) Compositions, growth mechanisms, and temporal relations of hydrothermal sulfide-sulfate-silica chimneys at the northern Cleft segment, Juan de Fuca Ridge. *Journal of Geophysical Research* **99**:4813-4832.

Lebaron, P., Servais, P., Troussellier, M., Courties, C., Vives-Rego, J., Muyzer, G., Bernard, L., Guindulain, T., Schäfer, H., and Stackebrandt, E. (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. *Aquatic Microbial Ecology* **19**: 255–267.

L'Haridon, S., Cilia, V., Messner, P., Ragúenès, G., Gambacorta, A., Sleytr, U.B., Prieur, D., and Jeanthon C. (1998) *Desulfurobacterium thermolithotrophum* gen. nov., sp. nov., a novel autotrophic, sulphur-reducing bacterium isolated from a deep-sea hydrothermal vent. *International Journal of Systematic Bacteriology* **48**:701-711.

Little, S.A., Stolzenbach, K.D., and Grassle, F.J. (1988) Tidal current effects on temperature in diffuse hydrothermal flow: Guaymas Basin. *Geophysical Research Letters* **15**:1491-1494.

Lloyd, K.G., Edgcomb, V.P., Molyneaux, S.J., Boer, S., Wirsen, C.O., Atkins, M.S., and Teske, A. (2005) Effects of dissolved sulfide, pH, and temperature on growth and survival of marine hyperthermophilic Archaea. *Applied and Environmental Microbiology* **71**:6383-6387.

Lonsdale, P. (1977) Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep-Sea Research* **24:**857-863.

López-García, P., Duperron, S., Philippot, P., Foriel, J., Susini, J., and Moreira, D. (2003) Bacterial diversity in hydrothermal sediment and epsilonproteobacterial dominance in experimental microcolonizers at the Mid-Atlantic Ridge. *Environmental Microbiology* **5**:961-976.

Lyautey, E., Jackson, C.R., Cayrou, J., Rols, J.L., and Garabétian, F. (2005) Bacterial community succession in natural river biofilm assemblages. *Microbial Ecology* **50**:589-601.

Martens, C.S. (1990) Generation of short chain organic acid anions in hydrothermally altered sediments of the Guaymas Basin, Gulf of California. *Applied Geochemistry* **5**:71-76.

Marteinsson, V.T., Moulin, P., Birrien, J., Gambacorta, A., Vernet, M., and Prieur D. (1997) Physiological responses to stress conditions and barophilic behavior of the hyperthermophilic vent archaeon *Pyrococcus abyssi. Applied and Environmental Microbiology* **63**:1230-1231.

Martinez, F., Taylor, B., Baker, E.T., Resing, J.A., and Walker, S. (2006) Opposing trends in crustal thickness and spreading rate along the back-arc Eastern Lau Spreading Center: implications for controls on ridge morphology, faulting, and hydrothermal activity. *Earth and Planetary Science Letters* **245**: 655-672.

Massana, R., DeLong, E.F., and Pedros-Alio, C. (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Applied and Environmental Microbiology* **66**:1777-1787.

Mehta, M.P., Butterfield, D.A., and Baross, J.A. (2003) Phylogenetic diversity of nitrogenase (nifH) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca Ridge. *Applied and Environmental Microbiology* **69**:960-970.

Mehta, M.P., Huber, J.A., and Baross, J.A. (2005) Incidence of novel and potentially archaeal nitrogenase genes in the deep Northeast Pacific Ocean. *Environmental Microbiology* **7**:1525-1534.

Mehta, M.P., and Baross, J.A. (2006) Nitrogen fixation at 92 degrees C by a hydrothermal vent archaeon. *Science* **314**:1783-1786.

McCliment, E.A., Voglesonger, K.M., O'Day, P.A., Dunn, E.E., Holloway, J.R., and Cary S.C. (2006) Colonization of nascent, deep-sea hydrothermal vents by a novel archaeal and nanoarchaeal assemblage. *Environmental Microbiology* **8**:114-125.

McCollom, T.M., and Shock, E.L. (1997) Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems. *Geochimica et Cosmochimica Acta* **61**:4375-4391.

McCollom, TM. (2007) Geochemical constraints on sources of metabolic energy for chemolithoautotrophy in ultramafic-hosted deep-sea hydrothermal systems. *Astrobiology* **7**:933-950.

Michael, P., and Seewald, J.S. (2007) Focus on: studies at the Lau Basin. In *Ridge 2000 events, Summer 2007.*

Miller, J.F., Shah, N.N., Nelson, C.M., Ludlow, J.M., and Clark, D.S. (1988) Pressure and temperature effects on growth and methane production of the extreme thermophile *Methanococcus jannaschii. Applied and Environmental Microbiology* **54**:3039-3042.

Miroshnichenko, M.L., Kostrikina, N.A., L'Haridon, S., Jeanthon, C., Hippe, H., Stackebrandt, E., and Bonch-Osmolovskaya, E.A. (2002) *Nautilia lithotrophica* gen. nov., sp. nov., a thermophilic sulfur-reducing epsilon-proteobacterium isolated from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* **52**:1299-1304.

Miroshnichenko, M.L., L'Haridon, S., Schumann, P., Spring, S., Bonch-Osmolovskaya, E.A., Jeanthon, C., and Stackebrandt, E. (2004) *Caminibacter profundus* sp. nov., a novel thermophile of *Nautiliales* ord. nov. within the class 'Epsilonproteobacteria', isolated from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* **54**:41-45.

Miroshnichenko, M.L. (2004) Thermophilic microbial communities of deep-sea hydrothermal vents. *Microbiology* **73**:1-13.

Miroshnichenko, M.L., and Bonch-Osmolovskaya, E.A. (2006) Recent developments n the thermophilic microbiology of deep-sea vents. *Extremophiles* **10**:85-96.

Moyer, C.L., Dobbs, F.C., and Karl, D.M. (1994) Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Applied and Environmental Microbiology* **60**:871-789.

Moyer, C.L., Dobbs, F.C., and Karl, D.M. (1995) Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Applied and Environmental Microbiology* **61**:1555-1562.

Moyer, C.L., Tiedje, J.M., Dobbs, F.C., and Karl, D.M. (1998) Diversity of Archaea from Loihi Seamount, Hawaii. *Deep-sea Research II* **45**:303-317.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., et al. (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437:**376-380.

Martinez, F., Taylor, B., Baker, E.T., Resing, J.A., and Walker, S. (2006) Opposing trends in crustal thickness and spreading rate along the back-arc Eastern Lau Spreading Center: implications for controls on ridge morphology, faulting, and hydrothermal activity. *Earth and Planetary Science Letters* **245**: 655-672.

Nakagawa, T., Nakagawa, S., Inagaki, F., Takai, K., and Horikoshi, K. (2004a) Phylogenetic diversity of sulfate-reducing prokaryotes in active deep-sea

hydrothermal vent chimney structures. *FEMS Microbiology Letters* **232**:145-152.

Nakagawa, S., Takai, K., Horikoshi, K., and Sako, Y. (2004b) *Aeropyrum camini* sp nov., a strictly aerobic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. *International Journal of Systematic and Evolutionary Microbiology* **54**:329-335.

Nakagawa, S., Inagaki, F., Takai, K., Horikoshi, K., and Sako, Y. (2005a) *Thioreductor micantisoli* gen. nov., sp nov., a novel mesophilic, sulfur-reducing chemolithoautotroph within the *epsilon-Proteobacteria* isolated from hydrothermal sediments in the Mid-Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* **55**:599-605.

Nakagawa, S., Takai, K., Inagaki, F., Horikoshi, K., and Sako, Y. (2005b) *Nitratiruptor tergarcus* gen. nov., sp. nov. and *Nitratifractor salsuginis* gen. nov., sp. nov., nitrate-reducing chemolithoautotrophs of the ε-*Proteobacteria* isolated from a deep-sea hydrothermal system in the Mid-Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* **55**:925-933.

Nakagawa, S., Takai, K., Inagaki, F., Chiba, H., Ishibashi, J., Kataoka, S., Hirayama, H., Nunoura, T., Horikoshi, K., and Sako, Y. (2005c) Variability in microbial community and venting chemistry in a sediment-hosted backarc hydrothermal system: Impacts of subseafloor phase-separation. *FEMS Microbiology Ecology* **54**:141-155.

Nelson, D.C., Wirsen, C.O., and Jannasch, H.W. (1989) Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. *Applied and Environmental Microbiology* **55**:2909-2917.

Nemergut, D.R., Anderson, S.P., Cleveland, C.C., Martin, A.P., Miller, A.E., Seimon, A., and Schmidt, S.K. (2007) Microbial community succession in an unvegetated, recently deglaciated soil. *Microbial Ecology* **53**:110-122.

Nercessian, O., Reysenbach, A.L., Prieur, D., and Jeanthon, C. (2003) Archaeal diversity associated with *in situ*samplers deployed on hydrothermal vents on the East Pacific Rise (13 degrees N). *Environmental Microbiology* **5**:492-502.

Nercessian, O., Bienvenu, N., Moreira, D., Prieur, D., and Jeanthon C. (2005) Diversity of functional genes of methanogens, methanotrophs and sulfate reducers in deep-sea hydrothermal environments. *Environmental Microbiology* **7**:118-132.

Nicol, G.W., Tscherko, D., Embley, T.M., and Prosser, J.I. (2005) Primary succession of soil Crenarchaeota across a receding glacier foreland. *Environmental Microbiology* **7**:337-347.

Ng, W.L., Schummer, M., Cirisano, F.D., Baldwin, R.L., Karlan, B.Y., and Hood, L. (1996) High-throughput plasmid mini preparations facilitated by micro-mixing. *Nucleic Acids Research* **24**:5045-5047.

Olsen, G.J., Lane, D.J., Giovannoni, S.J., Pace, N.R., and Stahl, D.A. (1986) Microbial ecology and evolution: a ribosomal RNA approach. *Annual Reviews of Microbiology* **40:**337-365.

Ortmann, A.C., Lawrence, J.E., and Suttle, C.A. (2002) Lysogeny and lytic viral production during a bloom of the cyanobacterium *Synechococcus* spp. *Microbial Ecology* **43**:225-231.

Pace, N.R. (1997) A molecular view of microbial diversity and the biosphere. *Science* **276**:734-740.

Pace, N.R., Stahl, D.A., Lane, D.J., and Olsen, G.J. (1986) The analysis of natural microbial populations by ribosomal RNA sequences. *Advances in Microbial Ecology* **9:**1-55.

Pagé, A., Juniper, S.K., Olagnon, M., Alain, K., Desrosiers, G., Quérellou, J., and Cambon-Bonavita, M.A. (2004) Microbial diversity associated with *Paralvinella sunfincola* tube and the adjacent substratum on an active deep-sea vent chimney. *Geobiology* **2**:225-238.

Pagé, A., Tivey, M.K., Stakes, D.S., and Reysenbach, A.L. (2008) Temporal and spatial archaeal colonization of hydrothermal vent deposits. *Environmental Microbiology* **10**:874-884.

Pearce, J.A., and Stern, R.J. (2006) Origin of back-arc magmas: trace elements and isotopes perspectives. *In* Back-arc spreading systems: geological, biological, chemical, and physical interactions. American Geophysical Union. Washington, DC. p. 63-86.

Peter, J.M., and Scott, S.D. (1988) Mineralogy, composition, and fluid-inclusion microthermometry of seafloor hydrothermal deposits in the Southern Trough of Guaymas Basin, Gulf of California. *Canadian Mineralogist* **26**:567-587.

Perner, M., Kuever, J., Seifert, R., Pape, T., Koschinsky, A., Schmidt, K., Strauss, H., and Imhoff, J.F. (2007) The influence of ultramafic rocks on microbial communities at the Logatchev hydrothermal field, located 15 degrees N on the Mid-Atlantic Ridge. *FEMS Microbiology Ecology* **61**:97-109.

Polz, M.F., and Cavanaugh, C.M. (1998) Bias in template-to-product ratios in multitemplate PCR. *Applied and Environmental Microbiology* **64:**3724-3730.

Poulton, S.W., Fralick, P.W., and Canfield, D.E. (2004) The transition to a sulphidic ocean approximately 1.84 billion years ago. *Nature* **431**:173-177.

Posada, D., and Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**:817-818.

Proskurowski, G., Lilley, M.D., Seewald, J.S., Früh-Green, G.L., Olson, E.J., Lupton, J.E., Sylva, S.P., and Kelley, D.S. (2008) Abiogenic hydrocarbon production at lost city hydrothermal field. *Science* **319**:604-607.

Qiu, X., Wu, L., Huang, H., McDonel, P.E., Palumbo, A.V., Tiedje, J.M., and Zhou, J. (2001) Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Applied and Environmental Microbiology* **67:**880-887.

Raskin, L., Rittmann, B.E., and Stahl, D.A. (1996) Competition and coexistence of sulfate-reducing and methanogenic populations in anaerobic biofilms. *Applied and Environmental Microbiology* **62**:3847-3857.

Reysenbach, A.L., Longnecker, K., and Kirshtein, J. (2000) Novel bacterial and archaeal lineages from an *in situ* growth chamber deployed at a Mid-Atlantic

Ridge hydrothermal vent. *Applied and Environmental Microbiology* **66**:3798-3806.

Reysenbach, A.L., Gotz, D., and Yernool, D. (2002) Microbial diversity of marine and terrestrial thermal springs. *In* Biodiversity of microbial life. J. T. Staley and A. Reysenbach (eds). Wiley-Liss. New York, NY. p. 345-421.

Reysenbach, A.L., Liu, Y., Banta, A.B., Beveridge, T.J., Kirshtein, J.D., Schouten, S., Tivey, M.K., Von Damm, K.L., and Voytek, M.A. (2006) A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. *Nature* **442**:444-447.

Rondon, M.R., August, P.R., Bettermann, A.D., Brady, S.F., Grossman, T.H., Liles, M.R., Loiacono, K.A., Lynch, B.A., MacNeil, I.A., Minor, C., Tiong, C.L., Gilman, M., Osburne, M.S., Clardy, J., Handelsman, J., Goodman, R.M. (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Applied and Environmental Microbiology* **66**:2541-2547.

Ruby, E.G., Wirsen. C.O., and Jannasch, H.W. (1981) Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos Rift hydrothermal vents. *Applied and Environmental Microbiology* **42**:317-342.

Ruby, E.G., and Jannasch, H.W. (1982) Physiological characteristics of *Thiomicrospira* sp. strain L-12 isolated from deep-sea hydrothermal vents. *Journal of Bacteriology* **149**:161-165.

Santegoeds, C.M., Ferdelman, T.G., Muyzer, G., and de Beer, D. (1998) Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. *Applied and Environmental Microbiology* **64**:3731-3739.

Schlesinger (1997) Biogeochemistry: an analysis of global change. Academic Press, New York, NY. 588 pages.

Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied and Environmental Microbiology* **71**:1501-1506.

Schloss, P.D., and Handelsman, J. (2006a) Introducing TreeClimber, a test to compare microbial community structures. *Applied and Environmental Microbiology* **72**:2379-2384.

Schloss, P.D., and Handelsman, J. (2006b) Introducing SONS, a tool for operational taxonomic unit-based comparisons of microbial community memberships and structures. *Applied and Environmental Microbiology* **72**:6773-6779.

Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N., Meyer, A.F., and Martin, A.M. (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* **88**:1379-1385.

Shock, E.L. (1992) Chemical Environments of Submarine Hydrothermal Systems. *Origins of Life and Evolution of the Biosphere* **22**:67-107.

Shock, E.L., McCollom, T.M., and Schulte, M.D. (1998) The emergence of metabolism from within hydrothermal systems. *In* Thermophiles: a key to molecular evolution and the origin of life? J. Wiegel and M.W.W. Adams (eds). Taylor & Francis. Philadelphia, PA. p. 59-76.

Schrenk, M.O., Kelley, D.S., Delaney, J.R. and Baross, J.A. (2003) Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and Environmental Microbiology* **69**:3580-3592.

Schrenk, M.O., Kelley, D.S., Bolton, S.A., and Baross, J.A. (2004) Low archaeal diversity linked to subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. *Environmental Microbiology* **6**:1086-1095.

Seewald, J.S., Cruse, A.M., Lilley, M.D. and Olsen, E.J. (1998) Hot-spring fluid chemistry at Guaymas Basin, Gulf of California: temporal variations and volatile content. *Eos* **79**:F46.

Seewald, J., McCollom, T., Proskurowski, G., Reeves, E., Mottl, M., Sharkey, C.G., Wheat, G., and Tivey, M. (2005) Aqueous volatiles in Lau Basin hydrothermal fluids. *EOS* **86**: abstract T31A-0479.

Seyfried, W.E., and Mottl, M.J. (1995) Geologic setting and chemistry of deep-sea hydrothermal vents. *In* The microbiology of deep-sea hydrothermal vents. D.M. Karl (ed). CRC Press. Boca Raton, FL. p. 1-34.

Shank, T.M., Fornari, D.J., Von Damm, K.L., Lilley, M.D., Haymon, R.M., and R.A. Lutz (1998) Temporal and spatial patterns of biological community

development at nascent deep-sea hydrothermal vents along the East Pacific Rise. *Deep-Sea Research,* **45**:465-515.

Shanks, W.C., Bischoff, J.L., and Rosenbauer, R.J. (1981) Seawater sulfate reduction and sulfur isotope fractionation in basaltic systems: interactions of seawater with fayalite and magnetite at 200-350°C. *Geochimica et Cosmochimica Acta* **45**:1977-1996.

Sipos, R., Székely, A.J., Palatinszky, M., Révész, S., Márialigeti, K., and Nikolausz, M. (2007) Effect of primer mismatch, annealing temperature and PCR cycle number on 16S rRNA gene-targetting bacterial community analysis. *FEMS Microbiology Ecology* **60:**341-345

Smith, G.P., Wiens, D.A., Fischer, K.M., Dorman, L.M., Webb, S.C., and Hildebrand, J.A. (2001) A complex pattern of mantle flow in the Lau Backarc. *Science* **292**:713-716.

Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., Arrieta, J.M., and Herndl, G.J. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the USA* **103**:12115-12120.

Somerville, C.C., Knight, I., Straube, W., and Colwell, R. (1989) Simple, rapid method for direct isolation of nucleic acids from aquatic environments. *Applied and Environmental Microbiology* **55**:548-554.

Spiess, F.N., Macdonald, K.C., Atwater, T., and Ballard, R., et al. (1980) East Pacific Rise: hot springs and geophysical experiments. *Science* **207**:1421-1433.

Straube, W.L., Deming, J.W., Somerville, C.C., Colwell, R.R., and Baross, J.A. (1990) Particulate DNA in Smoker Fluids: Evidence for Existence of Microbial Populations in Hot Hydrothermal Systems. *Applied and Environmental Microbiology* **56**:1440-1447.

Sullivan, J., Abdo, Z., Joyce, P., and Swofford, D.L. (2005) Evaluating the performance of a successive-approximations approach to parameter optimization in maximum-likelihood phylogeny estimation. *Molecular Biology and Evolution* **22**:1386-1392.

Suzuki, M., Rappé, M.S., and Giovannoni, S.J. (1998) Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Applied and Environmental Microbiology* **64:**4522-4529.

Swofford, D. L. (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10b. Sinauer Associates, Sunderland, Mass.

Takai, K., and Horikoshi, K. (1999) Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics* **152**:1285-1297.

Takai, K., Komatsu, T., Inagaki, F., and Horikoshi, K. (2001) Distribution of archaea in a black smoker chimney structure. *Applied and Environmental Microbiology* **67**:3618-3629.

Takai, K., Oida, H., Suzuki, Y., Hirayama, H., Nakagawa, S., Nunoura, T., Inagaki, F., Nealson, K.H., and Horikoshi, K. (2004a) Spatial distribution of marine crenarchaeota group I in the vicinity of deep-sea hydrothermal systems. *Applied and Environmental Microbiology* **70**:2404-2413.

Takai, K., Gamo, T., Tsunogai, U., Nakayama, N., Hirayama, H., Nealson, K.H., and Horikoshi, K. (2004b) Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea hydrothermal field. *Extremophiles* **8**:269-282.

Takai, K., Nealson, K.H., and Horikoshi. K. (2004c) *Hydrogenimonas thermophila* gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing chemolithoautotroph within the *epsilon-Proteobacteria*, isolated from a black smoker in a Central Indian Ridge hydrothermal field. *International Journal of Systematic and Evolutionary Microbiology* **54**:25-32.

Takai, K., Hirayama, H., Nakagawa, T., Suzuki, Y., Nealson, K.H., and Horikoshi, K. (2005a) *Lebetimonas acidiphila* gen. nov., sp. nov., a novel thermophilic, acidophilic, hydrogen-oxidizing chemolithoautotroph within the *'Epsilonproteobacteria'*, isolated from a deep-sea hydrothermal fumarole in the Mariana Arc. *International Journal of Systematic and Evolutionary Microbiology* **55**:183-189.

Takai, K., Campbell, B.J., Cary, S.C., Suzuki, M., Oida, H., Nunoura, T., Hirayama, H., Nakagawa, S., Suzuki, Y., Inagaki, F., and Horikoshi, K.

(2005b) Enzymatic and genetic characterization of carbon and energy metabolisms by deep-sea hydrothermal chemolithoautotrophic isolates of Epsilonproteobacteria. *Applied and Environmental Microbiology* **71**:7310-7320.

Takai, K., Suzuki, M., Nakagawa, S., Miyazaki, M., Suzuki, Y., Inagaki, F., and Horikoshi, K. (2006a) *Sulfurimonas paralvinellae* sp. nov., a novel mesophilic, hydrogen- and sulfur-oxidizing chemolithoautotroph within the *Epsilonproteobacteria* isolated from a deep-sea hydrothermal vent polychaete nest, reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans* comb. nov. and emended description of the genus *Sulfurimonas*. *International Journal of Systematic and Evolutionary Microbiology* **56**:1725-1733.

Takai, K., Nakagawa, S., Reysenbach, A.L., and Hoek, J. (2006b) Microbial ecology of mid-ocean ridges and back-arc basins. *In* Back-arc spreading systems: geological, biological, chemical, and physical interactions. Christie, D.M., Fisher, C.R., Lee, S.M., and Givens, S. (eds). American geophysical Union, Washington, DC. p. 185-213.

Taylor, C.D., Wirsen, C.O., and Gaill, F. (1999) Rapid microbial production of filamentous sulfur mats at hydrothermal vents. *Applied and Environmental Microbiology* **65**:2253-2255.

Teske, A., Hinrichs, K.U., Edgcomb, V., Gomez, A.D., Kysela, D., Sylva, S.P., Sogin, M.L., and Jannasch, H.W. (2002) Microbial diversity of hydrothermal

sediments in the Guaymas Basin: Evidence for anaerobic methanotrophic communities. *Applied and Environmental Microbiology* **68**:1994-2007.

Tivey, M.K., and J.R. Delaney (1986) Growth of large sulfide structures on the Endeavour Segment of the Juan de Fuca Ridge. *Earth and Planetary Science Letters* **77**:303-317.

Tivey, M.K., and McDuff, R.E. (1990a) Mineral precipitation in the walls of black smoker chimneys; a quantitative model of transport and chemical reaction. *Journal of Geophysical Research* **95**:12617-12637.

Tivey, M.K., Olson, L.O., Miller, V.W., and Light, R.D. (1990b) Temperature measurements during initiation and growth of a black smoker chimney. *Nature* **346**:51-54.

Tivey, M.K. (1995) The influence of hydrothermal fluid composition and advection rates on black smoker chimney mineralogy: Insights from modeling transport and reaction. *Geochimica et Cosmochimica Acta* **59**, 1933-1949.

Tivey, M.K., Bradley, A.M., Joyce, T.M., and Kadko, D. (2002) Insights into tide-related variability at seafloor hydrothermal vents from time-series temperature measurements. *Earth and Planetery Science Letters* **202**:693-707.

Tivey, M.K. (2004) Environmental conditions within active seafloor vent structures: sensitivity to fluid composition and fluid flow. *In* The subseafloor biosphere at mid-ocean ridges. Wilcock, W.S.D., DeLong, E.F., Kelley, D.S., Baross, J.A., and Cary, S.C. (eds). American Geophysical Union. Washington, DC. p. 137-152.

Trent, J.D., Osipiuk, J., and Pinkau, T. (1990) Acquired thermotolerance and heat shock in the extremely thermophilic archaebacterium *Sulfolobus* sp. strain B12. *Journal of Bacteriology* **172**:1478-1484.

Trent, J.D., Gabrielsen, M., Jensen, B., Neuhard, J., and Olsen J. (1994) Acquired thermotolerance and heat shock proteins in thermophiles from the three phylogenetic domains. *Journal of Bacteriology* **176**:6148-6152.

Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S., and Banfield JF. (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428**:37-43.

Varnes, E.S., Jakosky, B.M., and McCollom, T.M. (2003) Biological potential of Martian hydrothermal systems. *Astrobiology* **3**:407-414.

Von Damm, K.L., Edmond, J.M., Measures, C.I., and Grant, B. (1985) Chemistry of submarine hydrothermal solutions at Guaymas Basin, Golf of California. *Geochimica et Cosmochimica Acta* **49**:2221-2237.

Wachtershauser, G. (1988) Before enzymes and templates: theory of surface metabolism. *Microbiological Reviews* **52**:452-484.

Wachtershauser, G. (1990) Evolution of the first metabolic cycles. *Proceedings of the National Academy of Sciences of the U.S.A.* **87**:200-204.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* **173**:697-703.

Whitman, W.B. (2001) Family I. *Methanococcaceae. In* Bergey's Manual of Systematic Bacteriology. D.R. Boone, and R.W. Castenholtz (eds). Springer. New York, NY. p. 236-242.

Woese, C.R. (1987) Bacterial evolution. *Microbiological Reviews* **51:**221-271.

Woese, C.R. (1990) Evolutionary questions: the "progenote". *Science* **247**:789.

Woese, C.R. (1998) The universal ancestor. *Proceedings of the National Academy of Sciences of the U.S.A.* **95**:6854-6859.

Woese, C.R. (2007) The birth of the Archaea: a personal retrospective. In *Archaea: evolution, physiology, and molecular biology*. Garett, R.A., and Klenk, H.P. (eds). Blackwell Publishing. Malden, MA. p. 1-17.

Woese, C.R, and Fox, G.E. (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the U.S.A.* **74:**5088-5090.

Zellmer, K.E., and Taylor, B. (2001) A three-plate kinematic model for Lau Basin opening. *Geochemistry Geophysics Geosystems* **2**: 2000G000106.

APPENDIX A: Phylogenetic Analyses of Microbial 16S rRNA Genes Associated with Hydrothermal Deposits of Guaymas Basin

Summary of analyses

In addition to results presented in Chapter 1, supplementary phylogenetic analyses were conducted with 16S rRNA gene sequences obtained from Guaymas Basin chimneys. Sequences labeled BMX were obtained from a flange growing on the chimney that was present at Broken Mushroom before the deployments of thermocouple arrays. All samples were kept at -80˚C until processed in the laboratory.

Materials and Methods

Alignments of full-length 16S rRNA gene sequences were obtained using ARB (www.arb-home.de), with results of BLAST analyses and P. Hugenholtz's alignment (http://rdp.cme.msu.edu/misc/resources.jsp) as guides. Masks provided with P. Hugenholtz's ARB database were used as basis to select nucleotide positions for phylogenetic analyses. Only sections of 16S rRNA gene sequences that could be aligned unambiguously were used in phylogenetic analyses. PAUP* 4b10 (Swofford et al., 2002) was used for maximum likelihood analyses. Approximate models were first identified with ModelTest 3.7 (Posada and Crandall, 1998), using the Akaike Information Criterion (AIC). Heuristic tree searches with topological rearrangements of increasing complexity (nearest

neighbour interchange, subtree pruning and regrafting, tree bisection reconnection) were then conducted until trees with identical likelihood scores were found in two successive searches (Sullivan et al. 2005). Optimized rate matrices, shape parameters, and proportion of invariant sites were used to calculate bootstrap support (heuristic search with TBR, 100 replicates). A transition model of evolution with proportion of invariant sites and gamma distribution (TIM+I+G) was used in all analyses of *Archaea* and *Epsilonproteobacteria* 16S rRNA gene alignments (fig. 17, 18). A general time reversible model of evolution with proportion of invariant sites and gamma distribution (GTR+I+G) was used in all analyses of the 16S rRNA gene alignment for *Proteobacteria* and *Bacteria* (fig. 19, 20).

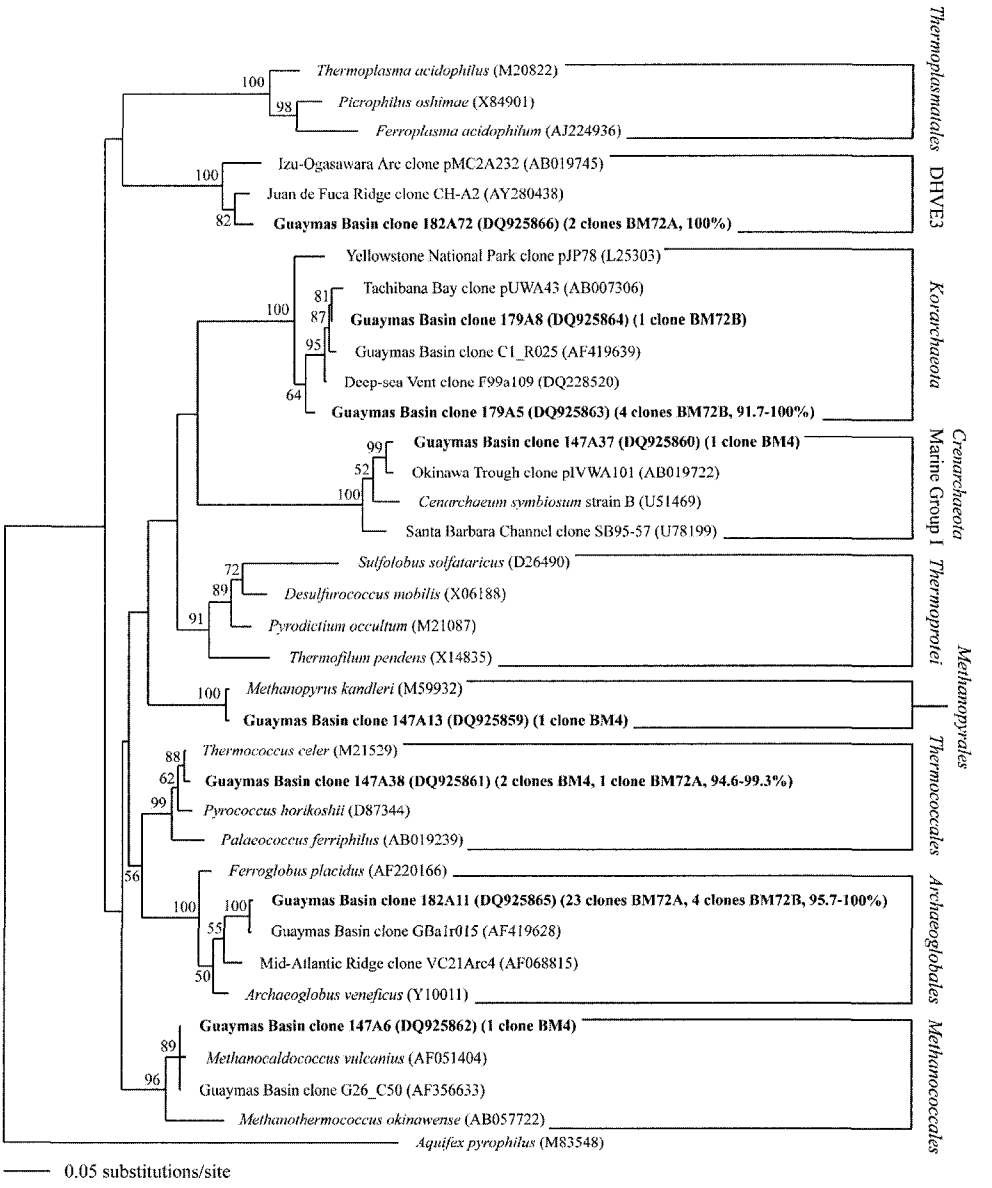

Figure 17. Phylogenetic relationship of archaeal 16S rRNA genes as determined by maximum-likelihood analysis. In parentheses, number of clones from each library represented by the phylotype, and percentages of similarity of the additional clones to this representative sequence (based on alignments performed with ARB and results by Moyer et al. (1996) for RFLP patterns). Bootstrap percentages are based on 1000 replicates (only higher than 50% shown).

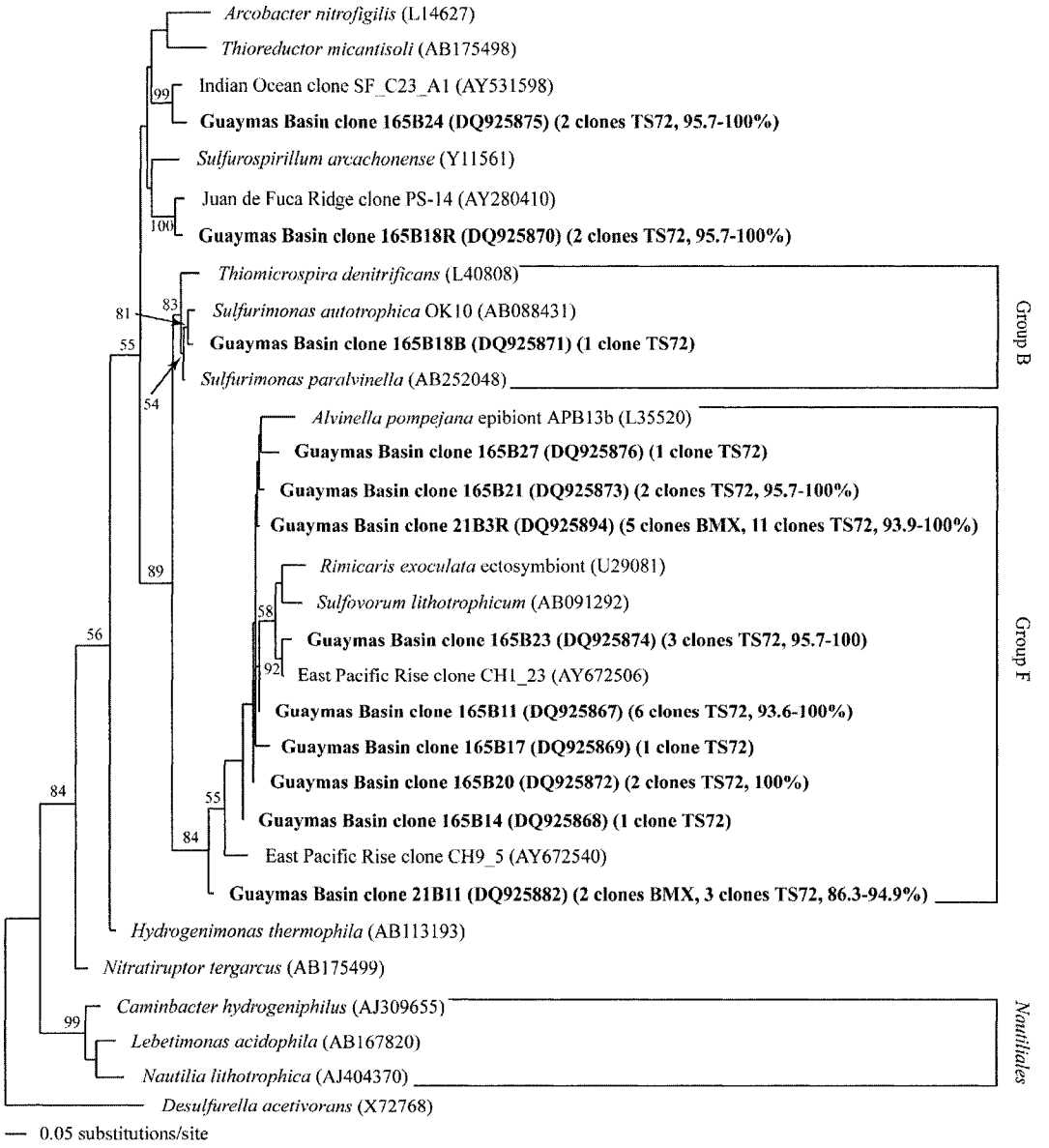

Figure 18. Phylogenetic relationship of epsilonproteobacterial 16S rRNA genes as determined by maximum-likelihood analysis. In parentheses, number of clones from each library represented by the phylotype, and percentages of similarity of the additional clones to this representative sequence (based on alignments performed with ARB and results by Moyer et al. (1996) for RFLP patterns). Bootstrap percentages are based on 1000 replicates (only higher than 50% shown).

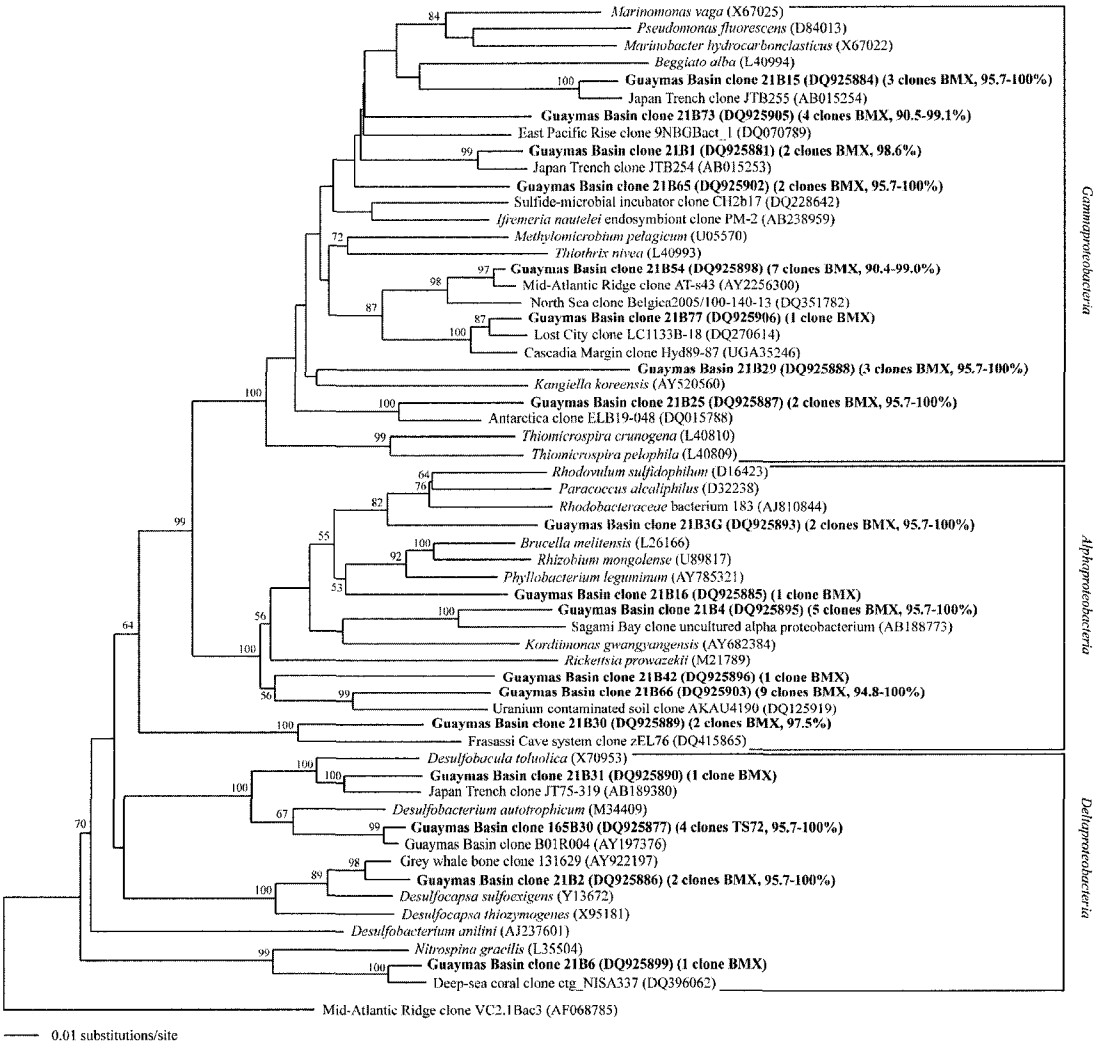

Figure 19. Phylogenetic relationship of proteobacterial 16S rRNA genes as determined by maximum-likelihood analysis. In parentheses, number of clones from each library represented by the phylotype, and percentages of similarity of the additional clones to this representative sequence (based on alignments performed with ARB and results by Moyer et al. (1996) for RFLP patterns). Bootstrap percentages are based on 1000 replicates (only higher than 50% shown).

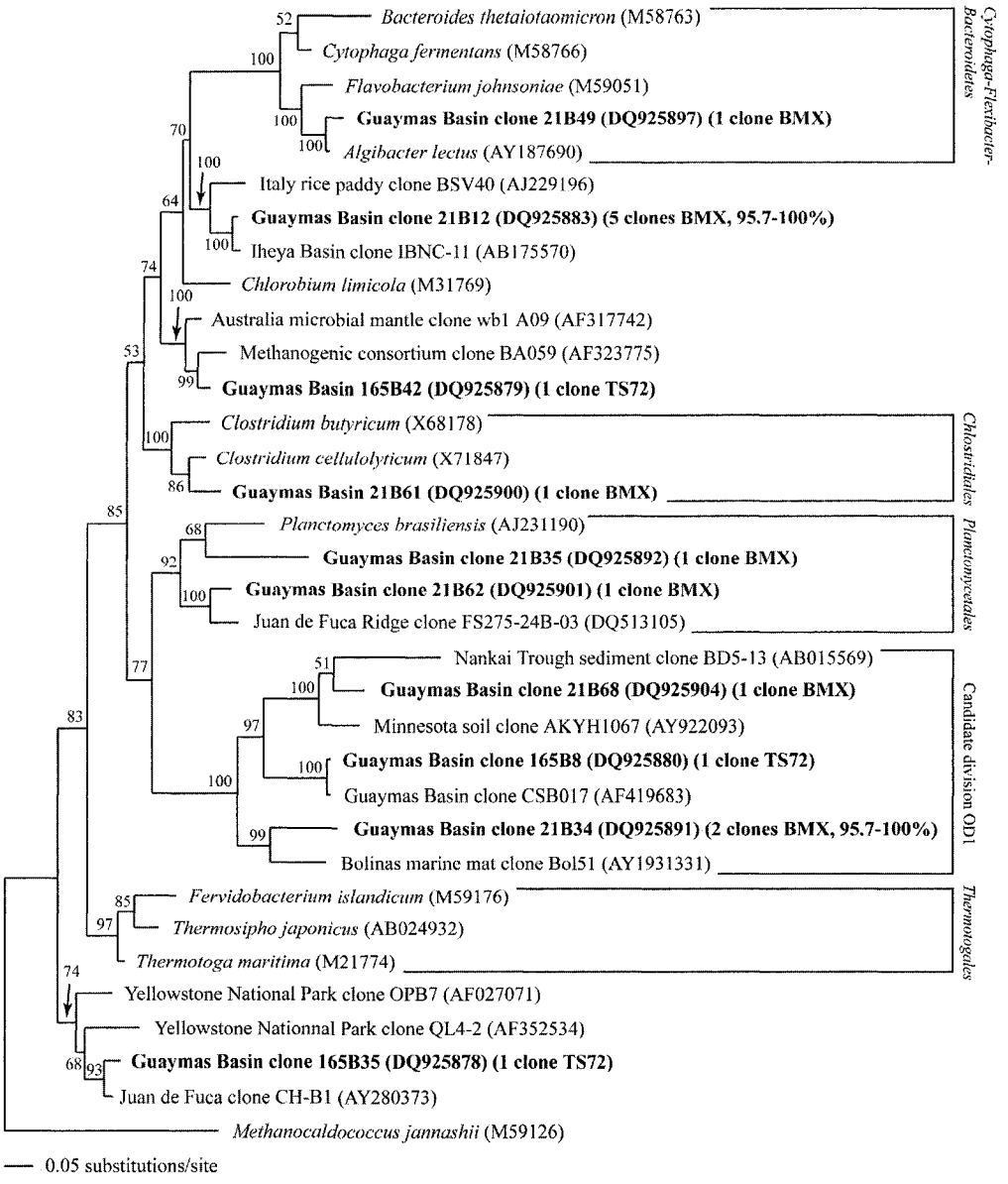

Figure 20. Phylogenetic relationship of bacterial 16S rRNA genes as determined by maximum-likelihood analysis. In parentheses, number of clones from each library represented by the phylotype, and percentages of similarity of the additional clones to this representative sequence (based on alignments performed with ARB and results by Moyer et al. (1996) for RFLP patterns). Bootstrap percentages are based on 1000 replicates (only higher than 50% shown).

APPENDIX B: Microbial Diversity Associated with Biofilms Exposed to Hydrothermal Fluids at the Galapagos Rift Vent Field

Summary of analyses

Since the historical discovery of active hydrothermal venting on the seafloor of the Galapagos Rift (86-89°W, 0.8°N) (Lonsdale 1977, Corliss et al. 1979), only a few research cruises have returned to this area. In 2001, we collected various samples from Rose Garden vent field (86°W) and Calyfield, on the western portion of the Rift (89.5°W). Samples included microbial mats (RB1, RB2, RB3, CF1, CF2, CF3, CF4), a basaltic rock (AG), and an extinct chimney (ECO1, ECO2, ECI1, ECI2) (Table V). All samples were kept at -80°C until processed in the lab.

Materials and Methods

Nucleic acids were extracted from samples RB2, RB3, CF3, and CF4 according to Somerville et al. (1989). DNA was extracted from the sulfide samples ECO1 and ECI1 using a modified version of Juniper et al. (2001) procedure. Nucleic acids were extracted from samples RB1, AG, CF1, CF2, ECO2 and ECI2 with the Ultraclean Soil DNA Kit (MoBio laboratories Inc., CA). Amplification of 16S rRNA gene fragments for denaturing gradient gel electrophoresis (DGGE) were separately performed with the bacteria-specific DGGE primer 338FGC (5'CGCC CGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCTCCTACGGGAGGC

AGCAG-3') and the universal primer 519R (5'ATTACCGCGGCTGCTGG-3'), and with the archaea-specific DGGE primer 344FGC (5'CGCCCGCCGCGCCCC GCGCCCGTCCCGCCGCCCCCGCCACGGGGCGCAGCAGGCGCGA-3') and the universal primer 519RP (5'GWATTACCGCGGCKGCTG-3'). One µl of betaine (Sigma-Aldrich CO., MO) was added in each 50 µl PCR reaction solution to prevent PCR inhibition by potential contaminants in the DNA preparation. Reactions were incubated in a thermal cycler under the following conditions: 5 min at 94˚C, followed by 30 cycles of 30 sec at 94˚C, 30 sec at 50˚C, 1.5 min at 72˚C, and a final 7 min at 72˚C. The PCR products were visualized by agarose gel electrophoresis. A nested PCR approach was used to obtain PCR products for samples RB3 (bacterial 16S rDNA amplification), and RB2, RB3, CF1, CF2 (archaeal 16S rDNA amplification). For these PCR reactions, 5 µl of the PCR products obtained from the earlier amplification were used in a 50 µl total reaction, with the same temperature cycle and PCR conditions (without betaine). Acrylamide gels (1X TAE: 40 mM Tris-acetate, 1 mM EDTA, pH 8.0, 6% acrylamide) containing a 30 to 70% gradient of urea and formamide were prepared and run in 1X TAE, with a DCODE apparatus (Bio-Rad Laboratories, CA) for 4h at 200V. Gels were stained with SYBR green (BioWhittaker Molecular Applications, ME) and visualized under UV (Fig. 21, 22). For sequencing, DNA from selected bands was collected with sterile pipette tips, and eluted in 25 µl of 10 mM Tris (pH 8.0). Five µl of the eluted DNA solutions were amplified using primers 338FGC and 519R for Bacteria, and 344FGC and 519RP

for Archaea. The temperature cycle and PCR conditions were the same as described earlier. Sequencing was performed with an ABI 3100-Avant sequencer, and the final sequences were submitted to a BLAST analysis against GenBank (Altschul *et al.*, 1990) (Table VI).

Table V. Presence of Archaea and Bacteria in samples collected at the Galapagos Rift.

Sample	Dive	Location	Description	Bacteria	Archaea
RB1	3789	Rosebud Vents (Mkr B)	Microbial mat	X	-
RB2	3790	Rosebud Vents (Mkr F)	Mat in tubeworm bush	X	X
RB3	3790	Rosebud Vents (Mkr F)	Mat in tubeworm bush	X	X
AG	3792	Axial Graben	Biofilm on Rock	X	X
CF1	3794	Calyfield (Mkr G)	Red microbial mat	X	X
CF2	3794	Calyfield (Mkr H)	Yellow microbial mat	X	X
CF3	3795	Calyfield (Mkr G)	Microbial mat	X	X
CF4	3795	Calyfield (Mkr G)	Microbial mat	X	X
ECO1	3795	Dead Chimney Field (Tgt 19)	Chimney (oxides)	X	-
ECO2	3795	Dead Chimney Field (Tgt 19)	Chimney (oxides)	X	-
ECI1	3795	Dead Chimney Field (Tgt 19)	Chimney (sulfides)	X	-
ECI2	3795	Dead Chimney Field (Tgt 19)	Chimney (sulfides)	X	-

Table VI. Summary of the phylotypes detected in all samples of the Galapagos Rift.

Band	Putative division	Closest Match	Accession no.	% Similarity
RB1-B1	γ-*Proteobacteria*	Mine *Pseudomonas* sp. strain GR-01	X86625	96
RB1-B2	γ-*Proteobacteria*	Marine *Pseudomonas* sp. K433	AF489289	96
RB1-B3	ε-*Proteobacteria*	Hydrothermal fluids clone 33-PA17B98	AF468697	95
RB1-B4	γ-*Proteobacteria*	Marine *Pseudomonas* sp. K433	AF489289	89
RB2-B1	ε-*Proteobacteria*	Hydrothermal fluids clone 33-FL74B00	AF468778	98
RB2-A1	Euryarchaeota (MGIII)	Deep-ocean clone DH148-W24	AF257278	98
RB3-B1	ε-*Proteobacteria*	Hydrothermal sediments clone B03P029	AY197410	95
RB3-B2	ε-*Proteobacteria*	Hydrothermal fluids clone 33-FL39B00	AF468781	93
RB3-B3	ε-*Proteobacteria*	Deep-sea vent chimney clone CHA3-30	AJ132732	94
RB3-A1	Euryarchaeota (MGIII)	Deep-ocean clone DH148-W24	AF257278	97
AG-B1	α-*Proteobacteria*	Ocean plankton clone Arctic 95B-2	AF355036	94
AG-A1	Crenarchaeota (MGI)	Deep-sea sediments clone CRA36-0 cm	AF119131	94
AG-A2	Crenarchaeota (MGI)	Deep-ocean clone ST-12K16A	AJ347776	97
CF1-B1	γ-*Proteobacteria*	Basalt DGGE band ODP-1156A-671	AY129847	94
CF1-B2	γ-*Proteobacteria*	Plankton *Pseudoalteromonas* sp. MED4	AF025547	98
CF1-B3	γ-*Proteobacteria*	Marine *Pseudomonas* sp. K433	AF489289	98
CF1-B4	γ-*Proteobacteria*	Basalt DGGE band ODP-1156A-671	AY129847	97
CF1-A1	Crenarchaeota (MGI)	Hydrothermal fluids clone 33-P18A00	AF355955	96
CF1-A2	Crenarchaeota (MGI)	Hydrothermal fluids clone 33-P81A00	AF355979	96
CF2-B1	γ-*Proteobacteria*	Basalt DGGE band ODP-1156A-671	AY129847	96
CF2-B2	γ-*Proteobacteria*	Basalt DGGE band ODP-1156A-671	AY129847	87

Table VI. *Continued.*

Band	Putative division	Closest Match	Accession no.	% Similarity
CF2-B3	γ-*Proteobacteria*	Plankton *Pseudoalteromonas* sp. MED4	AF025547	86
CF2-B4	γ-*Proteobacteria*	Basalt DGGE band ODP-1156A-671	AY129847	99
CF2-A1	Crenarchaeota (MGI)	Hydrothermal fluids clone 33-P18A00	AF355955	96
CF2-A2	Crenarchaeota (MGI)	Hydrothermal fluids clone 33-P124A99	AF355975	90
CF3-B1	ε-*Proteobacteria*	Deep-sea Vent Chimney clone CHA3-30	AJ132732	96
CF3-B2	ε-*Proteobacteria*	Hydrothermal sediments clone B03P029	AY197410	98
CF3-B3	ε-*Proteobacteria*	Deep-sea Vent Chimney clone CHA3-30	AJ132732	96
CF3-B4	ε-*Proteobacteria*	Deep-sea Vent clone bacterium Vent 21	AY075128	98
CF3-A1	Crenarchaeota (MGI)	Deep-sea sediments clone CRA36-0 cm	AF119131	97
CF3-A2	Crenarchaeota (MGI)	Deep-sea vent chimney clone FZ1aA2	AY166118	100
CF3-A3	Euryarchaeota (MGII)	Hydrothermal fluids clone 33-P39A00	AF355857	86
CF4-B1	ε-*Proteobacteria*	Hydrothermal fluids clone 33-FL70B00	AF468780	95
CF4-B2	ε-*Proteobacteria*	Cold seep clone CS037	AY279046	95
CF4-A1	Crenarchaeota (MGI)	Hydrothermal sampler clone pEPR853	AF526983	98
CF4-A2	Euryarchaeota	Saline pond clone 8-PML 8%	AF477919	88
ECO1-B1	δ-*Proteobacteria*	Forest wetland clone RCP1-85	AF523883	94
ECO2-B1	δ-*Proteobacteria*	Forest wetland clone RCP1-24	AF523882	92
ECO2-B2	γ-*Proteobacteria*	*Thialkalivibrio versutus*	AF126546	94
ECO2-B3	α-*Proteobacteria*	Marine clone CD2D5	AY038570	88
ECI1-B1	δ-*Proteobacteria*	Forest wetland clone RCP1-24	AF523882	88
ECI1-B2	CFB	Hydrothermal sampler VC2.1 Bac22	AF068798	93

Table VI. *Continued.*

Band	Putative division	Closest Match	Accession no.	% Similarity
ECI1-B3	CFB	Shallow hydrothermal vent clone ML-1f	AF208990	86
ECI1-B4	γ-*Proteobacteria*	*Thialkalivibrio versutus*	AF126546	93
ECI2-B1	CFB	Hydrothermal sampler VC2.1 Bac22	AF068798	78
ECI2-B2	*Firmicutes*	*Clostridium thermobutyricum*	X72868	95
ECI2-B3	CFB	Dead Deep-Sea Vent chimney	AB100013	81
ECI2-B4	γ-*Proteobacteria*	*Thialkalivibrio versutus*	AF126546	93

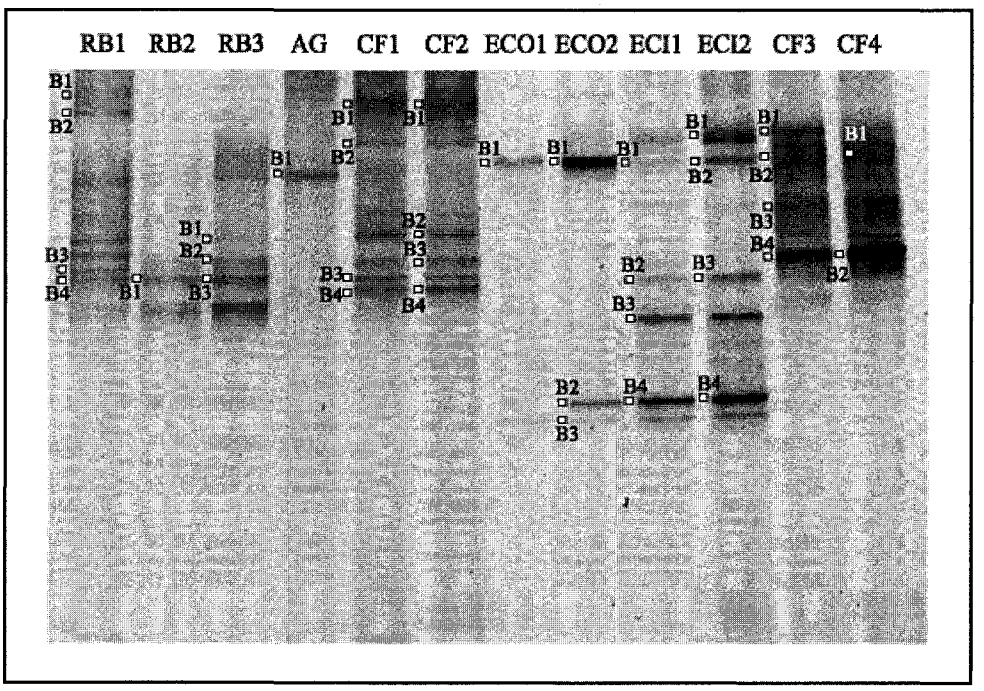

Figure 21. DGGE profiles of the Galapagos Rift samples (bacterial diversity).

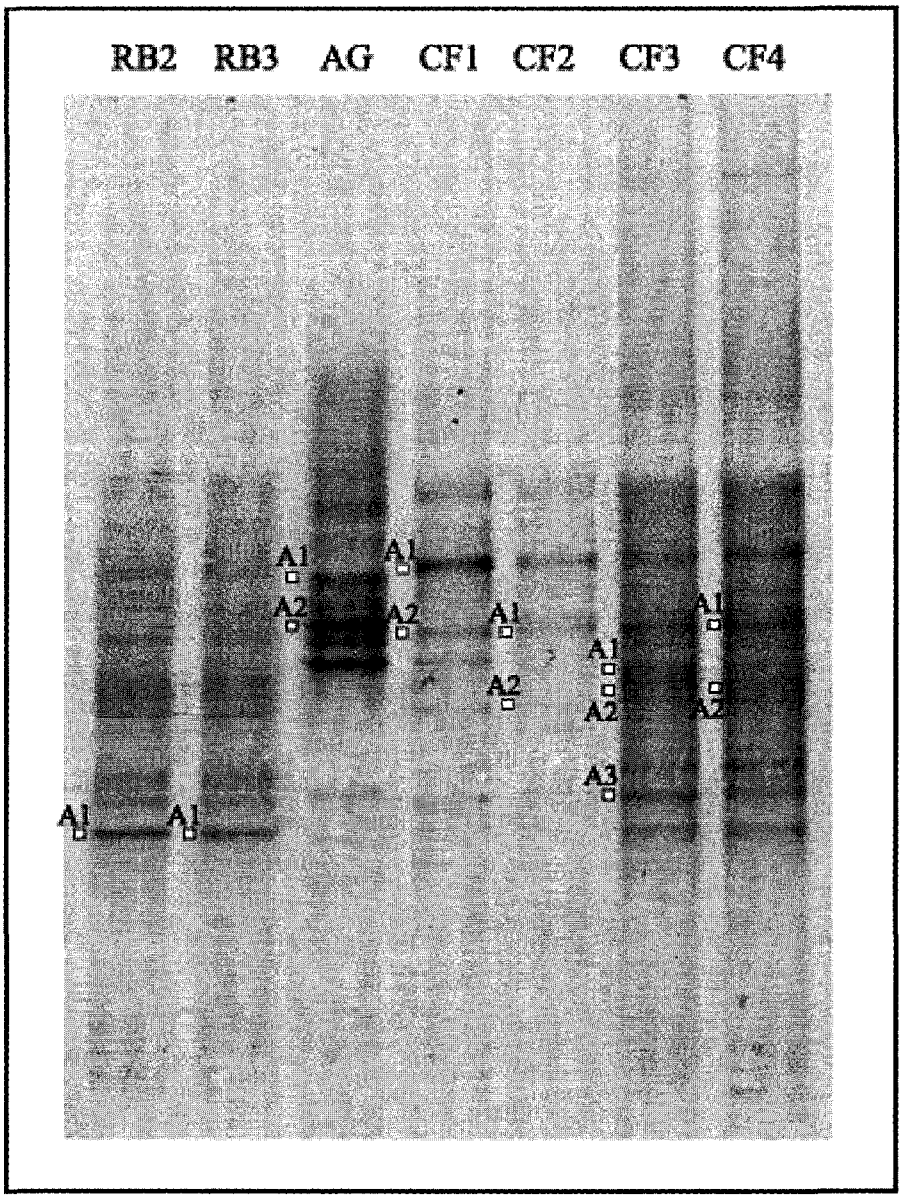

Figure 22. DGGE profiles of the Galapagos Rift samples (archaeal diversity). The sequenced bands are marked in red.

APPENDIX C: Microbial Diversity Associated with Hydrothermal Chimneys of the East Pacific Rise (9°N) Vent Field

Summary of analyses

In 2004, we collected samples of mineral samples from three active chimneys of the East Pacific Rise 9°N vent field (P Vent, Bio9', Q Vent) (9'50N, 104'17W) (Table VII). All samples were recovered from chimneys that emitted high temperature fluids (>341°), and stored at -80°C until processed in the lab.

Materials and Methods

Nucleic acids were extracted using the Ultraclean Soil DNA Kit (MoBio laboratories Inc., CA), and stored at -80°C until processed. Fragments of bacterial 16S rRNA genes for denaturing gradient gel electrophoresis (DGGE) were amplified using the bacteria-specific primer 338FGC (5'-CGCCCGCCGCGCCC CGCGCCCGTCCCGCCGCCCCCGCCCTCCTACGGGAGGCAGCAG-3'), and the universal primer 519R (5'-ATTACCGCGGCTGCTGG-3'). PCR mixes (50 µl final volume) contained 1X PCR buffer (2 mM $MgCl_2$, Promega, WI), 200 µM of each dNTP (Applied Biosystems, England), 200 nM of each forward and reverse primer, 2.5 U of *Taq* polymerase (Promega, WI), and 2 µl of betaine (Sigma-Aldrich CO., MO). The PCR conditions were as follow: 5 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 50°C, 30 sec at 72°C, and a final 7 min at 72°C. Fragments of archaeal 16S rRNA genes for DGGE were amplified using a

nested-PCR approach: Full-length 16S rRNA genes were first amplified using the archaea-specific primer 21F (5'-TCC GGTTGATCCTGCCRG-3') and the universal primer 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR mixes were as mentioned earlier. The PCR conditions were as follow: 5 min at 94°C, followed by 40 cycles of 30 sec at 94°C, 30 sec at 50°C, 1.5 min at 72°C, and a final 7 min at 72°C. One µl of the PCR products from each reaction was then added to a fresh PCR mix (49 µl) containing 200 nM of the archaeal-specific primer 344FGC (5'CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCACGGGGCC AGCAGGCGCGA-3') and the universal primer 519RP (5'-GWATTACCGCGGC KGCTG-3'), 1X PCR buffer, 200 µM of each dNTP, and 2.5 U of *Taq* polymerase. The new mixes were then incubated for 5 min at 94°C, followed by 40 cycles of 30 sec at 94°C, 30 sec at 50°C, 30 sec at 72°C, and a final 7 min at 72°C. An acrylamide gel (1X TAE: 40 mM Tris-acetate, 1 mM EDTA, pH 8.0, 6% acrylamide) containing a 30% to 70% gradient of urea and formamide was prepared, and the 16S rRNA gene fragments (approximately 50 ng per well) run in 1X TAE, with a DCODE apparatus (Bio-Rad Laboratories, CA), for 3.5 h at 200 V. The gel was stained with SYBR green (BioWhittaker Molecular Applications, ME) and visualized under UV (Fig. 23). DNA from selected bands was collected with sterile pipette tips, and eluted in 30 µl of 10 mM Tris (pH 8.0). Five µl of the eluted DNA solutions were added to PCR mixes containing the primers 338FGC and 519R for Bacteria, and 344FGC and 519RP for Archaea (mixes were as mentioned earlier, without betaine). The PCR conditions were as

described earlier. The PCR amplifications were confirmed by agarose gel electrophoresis and staining with ethidium bromide. Sequencing was performed with an ABI 3100-Avant sequencer, and the final sequences were submitted to a BLAST analysis against GenBank (Altschul *et al.*, 1990) (Table VIII, IX).

Table VII. Presence of Archaea and Bacteria in samples collected at 9°N on the East Pacific Rise.

Dive	Location	Description	Bacteria	Archaea
3961	P Vent	Black smoker (360°C fluids) - some oxides on surface	X	X
3964	Bio9'	Black smoker – covered by oxides	X	X
3972	Q Vent	Black smoker – potentially growth stage I	X	X

Table VIII. Archaeal phylotypes obtained in a DGGE analysis of 16S rRNA genes PCR-amplified from genomic DNA extracted of various chimney samples (East Pacific Rise).

Sample	Band	Putative division	Closest match	Accession no.	% Similarity
3961_1_2	A1	Uncult. Group I	Clone SM1	UAR296315	93 (29/31)
3961_1_7	A1	Uncult. Group I	Clone pMC2A17	AB019747	90 (30/33)
3961_1_7	A2	Uncult. Group I	Clone 19b-39	UAR294869	94 (32/34)
3964_1_1	A1	Aegean Sea-group	Clone 20c-54	UAR299202	91 (55/60)
3964_1_2	A1	Uncult. Group II	Clone VAL35-1	UEU131278	93 (40/43)
3964_1_3	A1, A2, A4	Aegean Sea-group	Clone 20c-42	UAR299198	86 (46/53)
3964_1_3	A3	Uncult. Group III	Clone ARR19	UAJ227932	88 (32/36)
3964_1_7	A1, A2, A3, A4	Uncult. Group II	Clone VAL35-1	UEU131278	93 (40/43) 96 (31/32)

Table VIII. *Continued.*

Sample	Band	Putative division	Closest match	Accession no.	% Similarity
3972_1_5	A1, A2, A3, A4, A5	Uncult. Group III	Clone 63-A23	UAR305083	92 (39/42) 96 (25/26)
3972_1_6	A1	*Methanomicrobiales*	Clone ABS22	UEY15397	91 (32/35)
3972_1_6	A2, A3, A4	DHVE2	Clone pMC2A10	AB019739	95 (91/95) 98 (98/100)

Table IX. Bacterial phylotypes obtained from a DGGE analysis of 16S rDNA amplified from chimney samples genomic DNA (East Pacific Rise).

Sample	Band	Putative division	Closest match	Accession no.	% Similarity
3964_1_1	B1	*Nitrospiraceae*	*Nitrospira* sp. SRI-237	AF255602	97 (83/85)
3964_1_3	B1	*Nitrospiraceae*	*Nitrospira* sp. SRI-237	AF255602	97 (82/84)
3964_1_7	B1	ε-group F	*Alvinella pompejana* epibiont 6C6	AY312990	95 (93/97)
3964_1_7	B2	ε-group D	*Caminibacter profundus*	CSP535664	92 (78/84)
3972_1_1	B1	*Acinetobacter sp.*	*Acinetobacter junii*	AF417863	99 (142/143)
3972_1_1	B2	*Acidovorax sp.*	*Acidovorax defluvii* strain PBWS3w90	AY682672	100 (101/101)
3972_1_1	B3	*Burkholderiales*	Clone pHAuB-34	AB072717	97 (138/142)
3972_1_5	B1	*Sulfurospirillum*-group	Proteobacterium BHI60-53	PRO431213	89 (116/130)
3972_1_5	B2	ε-group A	Clone P. palm C/A 26	UEP441213	96 (96/99)

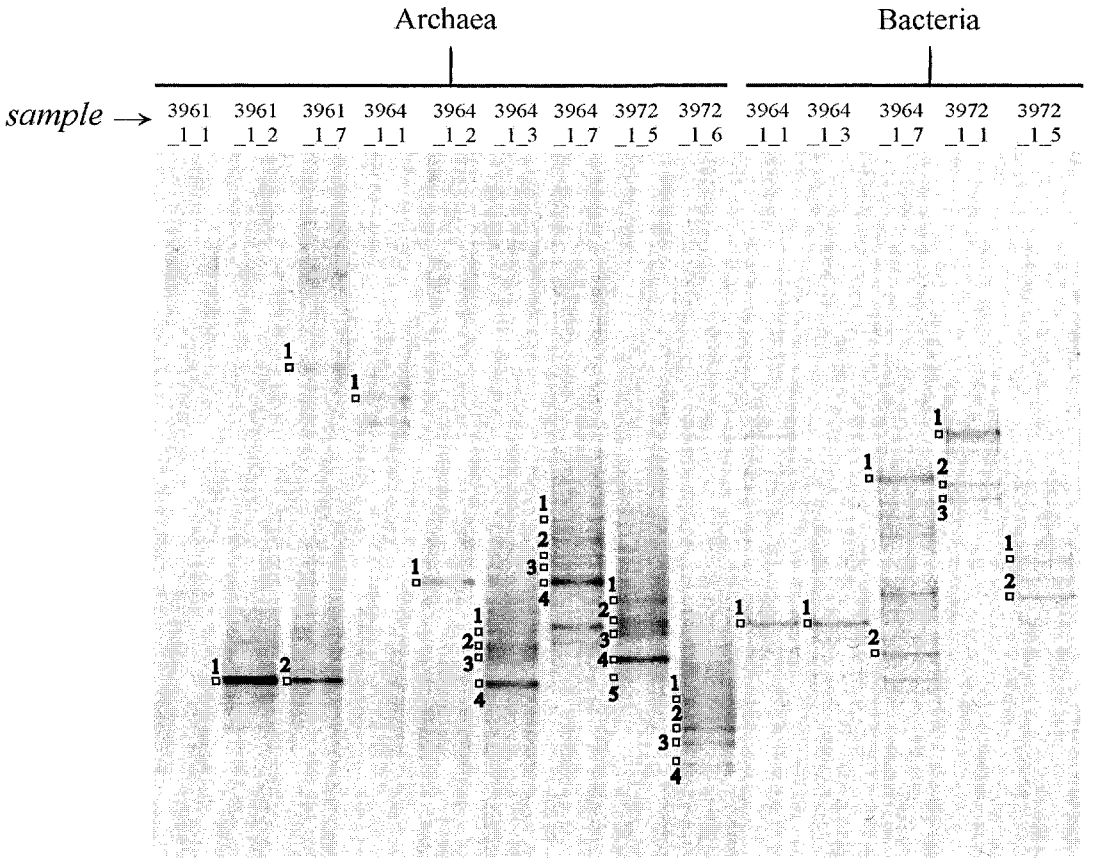

Figure 23. Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the 16S rRNA genes (archaeal and bacterial) amplified from subsamples of the chimney fragment collected at the East Pacific Rise 9°N vent field.