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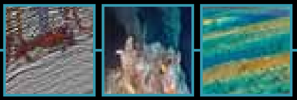

Chemoautotrophy at Deep-Sea Vents

Past, Present, and Future

BY STEFAN M. SIEVERT

AND COSTANTINO VETRIANI

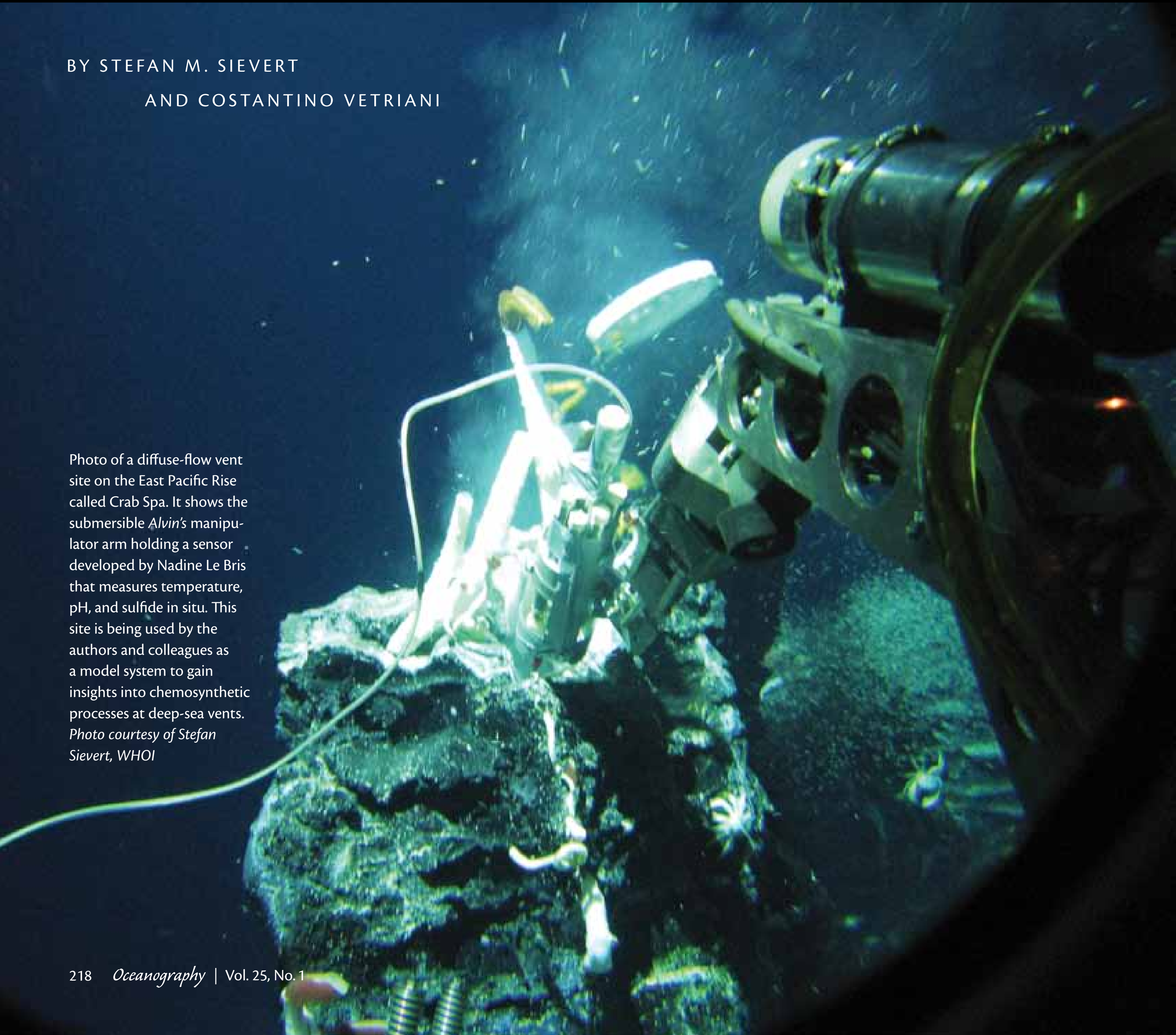

Photo of a diffuse-flow vent site on the East Pacific Rise called Crab Spa. It shows the submersible *Alvin's* manipulator arm holding a sensor developed by Nadine Le Bris that measures temperature, pH, and sulfide in situ. This site is being used by the authors and colleagues as a model system to gain insights into chemosynthetic processes at deep-sea vents. *Photo courtesy of Stefan Sievert, WHOI*

ABSTRACT. Chemolithoautotrophic microorganisms are at the nexus of hydrothermal systems by effectively transferring the energy from the geothermal source to the higher trophic levels. While the validity of this conceptual framework is well established at this point, there are still significant gaps in our understanding of the microbiology and biogeochemistry of deep-sea hydrothermal systems. Important questions in this regard are: (1) How much, at what rates, and where in the system is organic carbon being produced? (2) What are the dominant autotrophs, where do they reside, and what is the relative importance of free-swimming, biofilm-forming, and symbiotic microbes? (3) Which metabolic pathways are they using to conserve energy and to fix carbon? (4) How does community-wide gene expression in fluid and biofilm communities compare? and (5) How efficiently is the energy being utilized, transformed into biomass, and transferred to higher trophic levels? In particular, there is currently a notable lack of process-oriented studies that would allow an assessment of the larger role of these ecosystems in global biogeochemical cycles. By combining the presently available powerful "omic" and single-cell tools with thermodynamic modeling, experimental approaches, and new in situ instrumentation to measure rates and concentrations, it is now possible to bring our understanding of these truly fascinating ecosystems to a new level and to place them in a quantitative framework and thus a larger global context.

INTRODUCTION

The concept of chemolithoautotrophy (or chemosynthesis)—the production of organic matter from CO_2 coupled to a redox reaction between inorganic chemicals to obtain energy—dates to the work of Russian microbiologist Sergei Winogradsky, who discovered this process toward the end of the nineteenth century while studying the metabolism of sulfur-oxidizing and nitrifying bacteria. However, it took the discovery of deep-sea hydrothermal vents in 1977 on the Galápagos Rift (Lonsdale, 1977; Corliss et al., 1979) to identify an ecosystem where chemosynthesis—as opposed to photosynthesis—is the predominant form of organic carbon production (Jannasch and Wirsen, 1979; Jannasch and Mottl, 1985).

The discovery of deep-sea vents profoundly changed our view of life on Earth and made it abundantly clear that life could exist in the complete absence of light as the main source of energy. It is quite remarkable that we were able to send people to the moon and explore other stars within the universe before ecosystems solely based on chemosynthesis were discovered on our planet. However, the last 35 years or so have seen groundbreaking discoveries in deep-sea hydrothermal vent research, and the amount of knowledge obtained during this short amount of time is truly astonishing, especially considering the remoteness of the sites and the relatively modest amount of funds available for research. With the current Ridge 2000 Program coming to an end, it is timely to summarize our current knowledge in the area of deep-sea hydrothermal vent microbiology and biogeochemistry and to point a way forward to bring our understanding of these systems to a new level.

In a seminal review, Karl (1995) stated that "[c]omprehensive studies of the in situ metabolic processes of the various hydrothermal vent microbial assemblages have not been possible" and that "the quantitative importance of various electron-donor and acceptor pathways remains open to speculation." He goes so far as to say that in his view "the controversial chemolithoautotrophic hypothesis production has not been (rigorously) tested." We argue that although we now have a much better understanding of the composition and diversity of the microbial communities at deep-sea hydrothermal vents, and groundbreaking discoveries have been made (see Holden et al., 2012, in this issue), we have not fundamentally advanced in terms of determining the relevant metabolic pathways and functions, defining microbial activity, or measuring the relevant metabolic processes in situ. With new cultivation-independent methodologies, ranging from metagenomic and metaproteomic techniques to analyses at the single-cell level, it is now possible to address many of the unresolved questions, in particular, when they are applied simultaneously and in conjunction with geochemical measurements and experimental approaches. For this review, we will mainly focus on diffuse-flow vent microbial communities and the associated subseafloor environment.

We now know that deep-sea hydrothermal vents are located in a variety of

Stefan M. Sievert *(ssievert@whoi.edu) is Associate Scientist, Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA.* **Costantino Vetriani** *is Associate Professor, Department of Biochemistry and Microbiology and Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ, USA.*

submarine environments that include mid-ocean ridge and back-arc spreading centers, hotspot volcanoes, seamounts, and off-axis locations. Each of these environments is characterized by distinct differences in the discharged hydrothermal fluids and resulting biological communities (Reysenbach and Shock, 2002; Kelley et al., 2002; Schrenk et al., 2010). The hydrothermal fluids that form through high-temperature seawater-rock interactions are highly enriched in reduced chemical species such as H_2, H_2S, Fe^{2+}, or methane. The composition and concentration of these chemical species is strongly dependent on the geological setting, which again has a strong influence on the types of microbes inhabiting these systems (Kelley et al., 2002; Takai et al., 2006a; Amend et al., 2011). Mixing of these "geofuels" (Bach et al., 2006) with cold, oxygenated deep-sea water either above or below the seafloor creates chemical disequilibria that can be harnessed by metabolically versatile chemolithoautotrophic microorganisms, producing organic matter that forms the base of the food chains in these highly productive ecosystems. Thus, microbes effectively transfer energy from the geothermal source to higher trophic levels (Jannasch, 1995; Jannasch and Mottl, 1985). Although the validity of this conceptual framework is well established, there are still significant gaps in our understanding of the microbiology and biogeochemistry of deep-sea hydrothermal systems (Figure 1). These gaps include information describing the diversity of chemolithoautotrophic microorganisms that mediate critical reactions in different geothermal systems, the metabolic pathways used by microbes, rates of catalyzed reactions, amounts of organic carbon being produced, and the larger role of these ecosystems in global biogeochemical cycles.

Research over the last 30+ years has shown that aerobic and anaerobic chemolithoautotrophic microorganisms are abundant in a variety of mid-ocean ridge hydrothermal systems, in particular, methanogenic archaea and sulfur-oxidizing bacteria (e.g., Sievert et al., 2007; Takai and Nakamura, 2011; Table 1 shows some of the characteristics of chemoautotrophic organisms isolated as pure cultures from deep-sea hydrothermal vents). While initially sulfur-oxidizing *Gammaproteobacteria* were seen as important chemolithoautotrophs in diffuse-flow environments—mainly based on cultivation-based studies—recent research has brought bacteria belonging to the *Epsilonproteobacteria* and *Aquificales* to the forefront as important primary producers. They have been identified as significant members of the microbial communities at deep-sea hydrothermal vents, including free-living bacterial populations in vent fluids, on black smoker chimney walls, on surfaces exposed to hydrothermal fluids, and in the shallow subsurface (Reysenbach and Shock, 2002; Campbell et al., 2006; Nakagawa and Takai, 2008; Hügler and Sievert, 2011). For example, the microbial communities inhabiting the subseafloor at deep-sea vents are phylogenetically highly diverse (Huber et al., 2007), yet the predominant members of these communities are only distantly related to presently cultivated organisms, indicating that we have only scratched the surface of the metabolic potential and the extent of physiological diversity of the microorganisms inhabiting these environments. Following a volcanic eruption at Axial Volcano along the Juan de Fuca Ridge, it was further shown that the diversity among the *Epsilonproteobacteria* increased

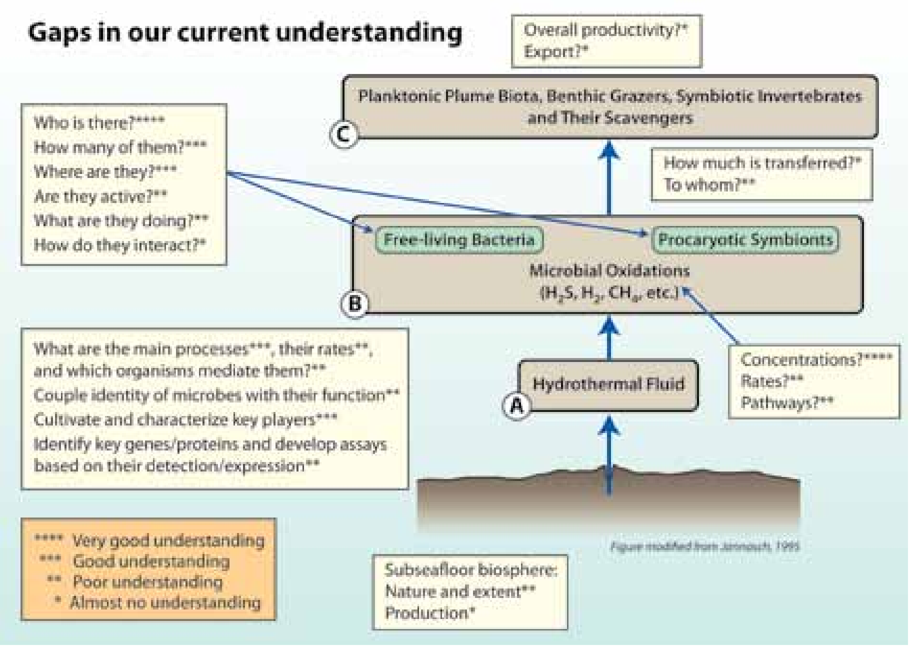

Figure 1. Subjective view of gaps in our current understanding of deep-sea hydrothermal systems as a guide for formulating research objectives.

Table 1. Characteristics of chemoautotrophic organisms isolated as pure cultures from deep-sea hydrothermal vents

	Isolation site	Optimum T (°C)	Electron donor(s)	Electron acceptor(s)	End product of nitrate respiration	Carbon source	Reference
Epsilonproteobacteria							
Sulfurovum lithotrophicum	MOT, Iheya, sediments	28–30	$S_2O_3^=$, S^0	NO_3^-, O_2	N_2	CO_2	Inagaki et al. (2004)
Sulfurimonas paralvinellae	MOT, Iheya, *Paralvinella*	30	H_2, $S_2O_3^=$, S^0	NO_3^-, O_2	N_2	CO_2	Takai et al. (2006b)
Sulfurimonas autotrophica	MOT, Hatoma Knoll, sediments	25	$S_2O_3^=$, S^0, H_2S	O_2		CO_2	Inagaki et al. (2003)
Thioreductor micantisoli	MOT, Iheya, sediments	32	H_2	NO_3^-, S^0	NH_4^+	CO_2	Nakagawa et al. (2005a)
Nautilia lithotrophica	EPR, 13°N, *Alvinella*	53	H_2, Formate	S^0		CO_2, Formate	Miroshnichenko et al. (2002)
Nautilia nitratireducens	EPR, 9°N, chimney	55	H_2, Formate, acetate, complex organic substrates	NO_3^-, S^0, $S_2O_3^=$, $SeO_4^=$	NH_4^+	CO_2, Formate	Pérez-Rodríguez et al. (2009)
Nautilia profundicola	EPR, 9°N, *Alvinella*	40	H_2, Formate	S^0		CO_2, Formate	Smith et al. (2008)
Nautili abyssi	EPR, 13°N, chimney	60	H_2	S^0		CO_2, Yeast Extract, Peptone	Alain et al. (2009)
Hydrogenimonas thermophila	CIR, Kairei Field, colonizer	55	H_2	NO_3^-, S^0, O_2	NH_4^+	CO_2	Takai et al. (2004c)
Nitratiruptor tergarcus	MOT, Iheya, chimney	55	H_2	NO_3^-, S^0, O_2	N_2	CO_2	Nakagawa et al. (2005b)
Nitratifractor salsuginis	MOT, Iheya, chimney	37	H_2	NO_3^-, O_2	N_2	CO_2	Nakagawa et al. (2005)
Caminibacter profundus	MAR, Rainbow, vent cap	55	H_2	NO_3^-, S^0, O_2	NH_4^+	CO_2	Miroshnichenko et al. (2004)
Caminibacter mediatlanticus	MAR, Rainbow, chimney	55	H_2	NO_3^-, S^0	NH_4^+	CO_2	Voordeckers et al. (2005)
Caminibacter hydrogeniphilus	EPR, 13°N, *Alvinella*	60	H_2	NO_3^-, S^0	NH_4^+	CO_2, complex organic substrates	Alain et al. (2002)
Lebetimonas acidiphila	Mariana Arc, colonizer	50	H_2	S^0		CO_2	Takai et al. (2005)
Aquificales							
Persephonella marina	EPR	73	H_2, $S_2O_3^=$, S^0	NO_3^-, S^0, O_2	N_2	CO_2	Götz et al. (2002)
Persephonella guaymasensis	Guaymas	70	H_2, $S_2O_3^=$, S^0	NO_3^-, O_2	N_2	CO_2	Götz et al. (2002)
Persephonella hydrogeniphila	Izu-Bonin Arc, Japan	70	H_2	NO_3^-, O_2	N_2	CO_2	Nakagawa et al. (2003)
Desulfurobacterium thermolithotrophum	MAR, Snake Pit, chimney	70	H_2	S^0, $SO_3^=$		CO_2	L'Haridon et al. (1998)
Desulfurobacterium crinifex	JdFR	60–65	H_2	NO_3^-, S^0	NH_4^+	CO_2	Alain et al. (2003)
Desulfurobacterium pacificum	EPR, 13°N, chimney	75	H_2	NO_3^-, S^0, $S_2O_3^=$	NH_4^+	CO_2	L'Haridon et al. (2006)
Desulfurobacterium atlanticum	MAR, 23°N, chimney	70–75	H_2	$S_2O_3^=$	NH_4^+	CO_2	L'Haridon et al. (2006)
Thermovibrio ammonificans	EPR 9°N, chimney	75	H_2	NO_3^-, S^0	NH_4^+	CO_2	Vetriani et al. (2004)
Thermovibrio guaymasensis	Guaymas, chimney	75–80	H_2	NO_3^-, S^0	NH_4^+	CO_2	L'Haridon et al. (2006)
Balnearium lithotrophicum	Izu-Bonin Arc, Japan	70–75	H_2	S^0		CO_2	Takai et al. (2003b)
Phorcysia thermohydrogeniphila	EPR, 13°N, colonizer, *Alvinella* tube	75	H_2	NO_3^-, S^0	NH_4^+	CO_2	Pérez-Rodríguez et al. (in press)
Hydrogenivirga okinawensis	SOT, Yonaguni Knoll IV	70–75	$S_2O_3^=$, S^0	NO_3^-, O_2	N_2	CO_2	Nunoura et al. (2008a)

MOT: Mid-Okinawa Trough; EPR: East Pacific Rise; CIR: Central Indian Ridge; MAR: Mid-Atlantic Ridge; JdFR: Juan de Fuca Ridge; SOT: Southern Okinawa Trough; TAG: Trans-Atlantic Geotraverse

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Table 1. Continued...

	Isolation site	Optimum T (°C)	Electron donor(s)	Electron acceptor(s)	End product of nitrate respiration	Carbon source	Reference
Gammaproteobacteria							
Thiomicrospira crunogena	EPR, 21°N, Vestimentiferan tube	28–32	$S_2O_3^=$, S^0, H_2S	O_2		CO_2	Jannasch et al. (1985)
Thiomicrospira thermophila	Mariana Arc, diffuse flow	35–40	$S_2O_3^=$, S^0, H_2S	O_2		CO_2, complex organic substrates	Takai et al. (2004a)
Salinisphaera hydrothermalis	EPR, 9°N, diffuse flow	30–35	$S_2O_3^=$, complex organic substrates	O_2		CO_2, *n*-alkanes, acetate, complex organic substrates	Crespo-Medina et al. (2009b)
Halothiobacillus hydrothermalis	Fiji Basin	35–40	$S_2O_3^=$, S^0, H_2S	O_2		CO_2, complex organic substrates	Durand et al. (1993)
Thioprofundum hispidum	Izu-Bonin Arc, Japan	39	$S_2O_3^=$, S^0, $S_4O_6^=$	NO_3^-, O_2	?	CO_2	Mori et al. (2011)
Thioprofundum lithotrophicum	MAR, TAG	50	$S_2O_3^=$, S^0, $S_4O_6^=$, $SO_3^=$	NO_3^-, O_2	?	CO_2	Takai et al. (2009)
Deferribacterales							
Deferribacter autotrophicus	MAR, Ashadze, chimney	60	H_2, complex organic substrates	Fe(III), Mn(IV), NO_3^-, S^0	NH_4^+	CO_2, complex organic substrates	Slobodkina et al. (2009)
Deferribacter abyssi	MAR, Rainbow, chimney	60	H_2, complex organic substrates	Fe(III), NO_3^-, S^0	NO_2^-	CO_2, complex organic substrates	Miroshnichenko et al. (2003)
Thermodesulfobacteriaceae and related taxa							
Thermodesulfobacterium hydrogeniphilum	Guaymas	75	H_2	$SO_4^=$		CO_2	Jeanthon et al. (2002)
Thermodesulfatator indicus	CIR, Kairei Field	70	H_2	$SO_4^=$		CO_2	Moussard et al. (2004)
Thermodesulfatator atlanticus	MAR, Rainbow	65–70	H_2	$SO_4^=$		CO_2, complex organic substrates	Alain et al., (2010)
Thermosulfidibacter takaii	SOT, Yonaguni Knoll IV	70	H_2	S^0		CO_2	Nunoura et al. (2008b)
Archaea							
Ignicoccus pacificus	EPR, 9°, Chimney	90	H_2	S^0		CO_2	Huber et al. (2000)
Geoglobus ahangari	Guaymas, chimney	88	H_2, complex organic substrates	Fe(III)		CO_2, complex organic substrates	Kashefi et al. (2002)
Methanothermococcus okinawanensis	MOT, Ilheya	60–65	H_2, Formate	CO_2		CO_2	Takai et al. (2002)
Methanocaldococcus indicus	CIR	85	H_2	CO_2		CO_2	L'Haridon et al. (2003)
Methanotorris formicicus	CIR	85	H_2, Formate	CO_2		CO_2	Takai et al. (2004b)
Methanopyrus kandleri	Guaymas	100	H_2	CO_2		CO_2	Kurr et al. (1991)
Methanopyrus kandleri strain 116	CIR, Kairei Field, colonizer on chimney	100; 105 under 20 MPa	H_2	CO_2		CO_2	Takai et al. (2008)
Pyrolobus fumarii	MAR	105	H_2	NO_3^-, S^0, O_2	NH_4^+	CO_2	Blöchl et al. (1997)
"Geogemma barossii"	JdFR	105–107; Max 121	H_2	Fe(III)		CO_2	Kashefi and Lovley (2003)
Archaeoglobus veneficus	MAR, chimney	75–80	H_2, organic acids, glucose, ethanol	S^0, $SO_3^=$		CO_2	Huber et al. (1997)
Archaeoglobus sulfaticallidus	Flank of JdFR, steel surface of a seafloor borehole observatory	75	H_2, pyruvate, lactate	$SO_4^=$, $S_2O_3^=$, $SO_3^=$		CO_2	Steinsbu et al. (2010)

MOT: Mid-Okinawa Trough; EPR: East Pacific Rise; CIR: Central Indian Ridge; MAR: Mid-Atlantic Ridge; JdFR: Juan de Fuca Ridge; SOT: Southern Okinawa Trough; TAG: Trans-Atlantic Geotraverse as the temperature of the vent fluids decreased, suggesting a tight coupling between physicochemical conditions and microbial community composition (Huber et al., 2003). On the other hand, it has been shown that microbial communities from different diffuse-flow sites, sometimes in close proximity, can be significantly different despite similar geochemical conditions (Opatkiewicz et al., 2009). At present, it is unknown whether the phylogenetic differences also have a functional counterpart.

A major breakthrough has been the cultivation of *Epsilonproteobacteria* and *Aquificales* from deep-sea hydrothermal vents subsequent to their detection by 16S rRNA-based surveys (Campbell et al., 2001; Götz et al., 2002; Takai et al., 2003a). Bringing them into culture has greatly aided elucidation of their ecological role and biogeochemical significance (Table 1). Based on these studies, *Epsilonproteobacteria* and *Aquificales* exhibit similar metabolisms—the oxidation of reduced sulfur compounds and/or hydrogen with both oxygen and nitrate, or the oxidation of hydrogen with elemental sulfur reduction coupled to the fixation of inorganic carbon—and thus rely on similar resources, but in different temperature regimes (*Epsilonproteobacteria:* 20–60°C; *Aquificales*: 60–80°C; Reysenbach and Shock, 2002; Campbell et al., 2006; Nakagawa and Takai, 2008; Hügler and Sievert, 2011). The success of these microorganisms at deep-sea vents has frequently been attributed to their metabolic versatility and the ability to use alternative electron donors (e.g., sulfide, hydrogen) and/or electron acceptors (e.g., oxygen, nitrate, sulfur), reflecting the dynamic environment in which they live (Campbell et al., 2006). However, little is known about the in situ activities of these organisms, the preferential or simultaneous use of alternative electron-donors/acceptors, and the conditions under which the corresponding enzymes are expressed. In particular, the high phylogenetic microdiversity revealed by 16S rRNA sequence analyses (e.g., Huber et al., 2007) poses the question: to what degree do the corresponding organisms share the same metabolism or perform different functions? Possibly, there is functional redundancy within the community, with different taxa carrying out similar functions using homologous pathways, but being optimally adapted to slightly different environmental conditions, like temperature. Overall, such strategy might impart robustness to the performance of the community and its response to the dynamic vent environment that is characterized by steep thermal and redox gradients, as well as frequent disturbances.

Two eruptive cycles on the East Pacific Rise (EPR) at 9°N further emphasized the critical role of microbial biofilms in the initial colonization of post-eruptive diffuse-flow vents. Following the 1991 eruption at this site, Shank et al. (1998) reported that "white filamentous microbial mats, 10 cm thick, blanketed up to 50 m^2 areas of fresh lava flows surrounding nascent venting areas." These observations were reinforced by more recent studies, which showed that chemosynthetic *Epsilonproteobacteria* dominated biofilm communities at various vent sites (Taylor et al., 1999; López-García et al., 2003; Alain et al., 2004; recent work of authors Sievert and Vetriani). At present, the specific mechanisms of microbial biofilm formation at diffuse-flow deep-sea vents are unknown.

FUNCTIONAL ANALYSES USING "OMIC" AND SINGLE-CELL APPROACHES

To address the questions posed above and to obtain a better understanding of the underlying processes and activities requires the integration of a variety of complementary techniques that target the whole community (bulk rates, meta-"omics"[1])—including active community members—as well as single cells (e.g., Halogen In Situ Hybridization-Secondary Ion Mass Spectroscopy, or HISH-SIMS; Musat et al., 2008). Metagenomic approaches make it possible to assess the gene content of the whole community without the need to grow and isolate the individual organisms in the laboratory (Gilbert and Dupont, 2011). The data obtained from these kinds of analyses provide an unprecedented view into the diversity, and, more importantly, the functional gene representation of the microbial communities being studied. With these data at hand, it is possible not only to determine which metabolic pathways are potentially present but also to assess their relative importance by analyzing frequencies of key genes indicative of these pathways. However, a major shortcoming of this approach is that the genes identified in the metagenome can only be linked to specific organisms by using bioinformatic approaches and not directly, as is possible with the genome

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[1] "omics" refer to fields of biological study ending in -omics, such as genomics or proteomics.

of an individual organism. However, it is now possible to obtain genomic information about single cells directly from the environment without the need to first cultivate them in the laboratory (Stepanauskas and Sieracki, 2007; Woyke et al., 2009). This method ideally complements the metagenomic approach described above, in which the genomic potential of the whole community, rather than individual cells, is investigated. In addition to the metagenome, one can look at the genes being transcribed in a microbial community (metatranscriptome) or directly at the expressed proteins (metaproteome): both approaches provide information on what kinds of organisms are actually active, and on the pathways being utilized at a particular point in time and under a particular set of conditions.

Applying these methods has already resulted in a number of breakthroughs that contributed critical information to better understand the role of marine microbes in biogeochemical cycling as well as their specific adaptations, in particular the photic zone (DeLong, 2009, and references therein; McCarren et al., 2010; Morris et al., 2010; Sowell et al., 2010), and, more recently, the deep sea (e.g., DeLong et al., 2006; Eloe et al., 2011; Swan et al., 2011). Functional approaches have already been applied with great success to studies of symbiotic bacteria of hydrothermal vent invertebrates, a less-diverse and more tractable system as compared to the more diverse free-living communities (e.g., Markert et al., 2007; Gryzmski et al., 2008; Hügler et al., 2011). As an outstanding recent example, the use of hydrogen oxidation as an energy source by a hydrothermal vent symbiosis was demonstrated for the first time by using an integrated approach involving metagenomic sequencing, single-gene fluorescent in situ hybridization (FISH), immunohistochemistry, shipboard incubations, and in situ mass spectroscopy (Petersen et al., 2011).

Recent work either comparing tubeworms of the same species at different sites or different species at the same sites (Gardebrecht et al., 2011; Robidart et al., 2011) builds upon earlier metaproteomic analyses carried out to gain insights into the metabolism and physiology of the uncultivated endosymbiont of the giant tube worm *Riftia pachyptila* (Markert et al., 2007, 2011). New insights into the microorganisms involved in these communities and the pathways they use illustrates the power of using metagenomic approaches to study microbial communities associated with hydrothermal vent chimneys (Brazelton and Baross, 2009, 2010; Xie et al., 2011). Interestingly, the metagenome of a microbial community from a black smoker chimney from the Mothra hydrothermal vent field on the Juan de Fuca Ridge in the Northeast Pacific Ocean clustered most closely with one obtained from a carbonate chimney at the Lost City hydrothermal vent field on the Mid-Atlantic Ridge, two environments with contrasting chemistry (Xie et al., 2011). Specific functions appear to be overrepresented in both communities, suggesting that growth as a biofilm in chimneys selects for certain traits irrespective of the geochemical conditions.

We are currently pursuing metatranscriptomic and metaproteomic studies of diffuse-flow vent microbial communities and associated biofilms, and first results clearly show the dominance of *Epsilonproteobacteria* as the active community members. These data corroborate earlier studies of the genetic potential of hydrothermal vent communities and provide direct evidence that chemosynthesis is the dominant metabolism of these communities. Our work indicates that the reductive TCA (tricarboxylic acid) cycle for carbon fixation and the SOX (sulfur oxidation) pathway and sulfide-quinone reductase pathway for the oxidation of reduced sulfur compounds are the dominant chemosynthetic pathways, providing an opportunity for comparisons with biochemical analyses on relevant pure cultures (Yamamoto et al., 2010). These findings add to the notion that chemoautotrophic production at deep-sea hydrothermal vents occurs to a large extent via carbon fixation pathways other than the well-known Calvin cycle (Hügler and Sievert, 2011). Further, from the variety of bioavailable electron acceptors in low-temperature mixing hydrothermal habitats, the role of seawater NO_3^- in supporting chemolithoautotrophic production has not been fully explored, despite the highly energetic nature of the microbial mediated nitrate reduction reactions (Jannasch and Mottl, 1985; Amend and Shock, 2001). However, recent data strongly suggest that nitrate reduction either to N_2 (denitrification) or to NH_4^+ (dissimilatory reduction of nitrate to ammonium, DNRA) might be responsible for a significant fraction of chemoautotrophic production. This conclusion is based on (1) the frequent isolation of chemolithoautotrophs that have the potential to couple the oxidation of reduced sulfur compounds and hydrogen to the reduction of nitrate (Table 1; Campbell et al., 2006; Nakagawa and Takai, 2008), (2) the nonconservative depletion of nitrate and accumulation of ammonia in diffuse-flow hydrothermal fluids (Butterfield et al., 2004), and (3) the presence of transcripts of the nitrate reductase genes and the strong expression of all enzymes involved in nitrate reduction to N_2 in the metaproteome of diffuse-flow hydrothermal vent communities obtained at 9°N EPR (recent work of the authors).

THERMODYNAMIC MODELS COUPLED WITH FUNCTIONAL ANALYSES

A very productive path toward increasing understanding of deep-sea vent microbial communities is the cross-iteration of thermodynamic models and functional-based analyses of microbial communities (see also Holden et al., 2012, in this issue). Which of the possible metabolisms predicted by models are actually present or even expressed? Are there pathways that are expressed but not predicted by the models? These kinds of approaches have already been successfully applied and have resulted in new insights into the drivers of microbial community composition and activity (Schmidt et al., 2008; Houghton and Seyfried, 2010; Amend et al., 2011; Flores et al., 2011; Roussel et al., 2011; Takai and Nakamura, 2011). In the future, the addition of "omic" approaches should increase the power of such analyses by also assessing the kinds of pathways present and expressed in the respective communities.

Thermodynamic model data can also serve as an important screening tool for identifying which kind of organisms to target for cultivation and what processes to target for further inquiries. For example, thermodynamic models simulating the step-by-step dilution of end-member hydrothermal fluid with seawater often display a sharp boundary between oxic and anoxic conditions that is dependent on the assumption that chemical equilibrium is achieved between $H_{2(aq)}$ and $O_{2(aq)}$ (McCollom and Shock, 1997; Shock and Holland, 2004). However, experimental data demonstrating inhibition of the abiotic oxidation of hydrogen (Knallgas reaction) at temperatures < 100°C suggest that $H_{2(aq)}$ and $O_{2(aq)}$ may be available to aerobic hydrogen-oxidizing bacteria in low temperature vents (Foustoukos et al., 2011). Furthermore, anaerobic hydrogen-oxidizing bacteria may also be active at temperatures lower than 40°C due to the persistence of $H_{2(aq)}$ in seawater/hydrothermal fluid mixtures (Foustoukos et al., 2011). Thus, under H_2-O_2 disequilibrium, the anoxic/oxic boundaries along seawater/hydrothermal fluid mixing interfaces may not be as sharp as predicted by thermodynamic models, and microbially mediated $H_{2(aq)}$ oxidation could provide one of the largest energy sources available at low-temperature diffuse-flow vent sites (Shock and Holland, 2004).

At present, however, the importance of hydrogen oxidation for chemoautotrophic production is poorly constrained. The fact that it is used by symbioses suggests that it is probably also widespread among free-living microbes, which is supported by recent studies using a combination of approaches, including thermodynamic modeling in conjunction with microbial diversity assessments, geochemical flux measurements, and/or incubation studies (Perner et al., 2010; Takai and Nakamura, 2011; Wankel et al., 2011). On the other hand, carefully designed experiments can also help to further constrain and refine thermodynamic models, for example, by determining growth parameters and growth efficiencies of pure cultures, defined co-cultures, or natural communities (e.g., Houghton et al., 2007). For example, ATP synthesis in denitrification is generally far lower than expected from free energy changes (Strohm et al., 2007), emphasizing the need to perform actual growth experiments to verify predictions based on thermodynamic calculations. The possibility of novel energy-yielding metabolisms not predicted by thermodynamic models exists as well (e.g., Nakamura et al., 2010).

RATE MEASUREMENTS

Since the early days of the discovery of deep-sea hydrothermal vents, efforts have been made to determine rates of chemoautotrophic production, a key parameter for estimating the productivity of these systems. In particular, Holger Jannasch and colleagues carried out a number of experiments (both at the seafloor and aboard ship) to measure chemoautotrophic production (e.g., Tuttle et al., 1983; Wirsen et al., 1986). These pioneering experiments showed very low rates in the range of 0.05 nmol C L^{-1} hr^{-1} for incubations conducted at an ambient deep-sea temperature of 3°C either at the seafloor or at atmospheric pressure. When incubations were conducted at atmospheric pressure and vent temperature of 23°C, higher rates of approximately 21 nmol C L^{-1} hr^{-1} were measured, suggesting that temperature, rather than pressure, may be the main factor that stimulated the activity of mesophilic chemoautotrophs. In one experiment conducted at in situ pressure in an isobaric sampler that was brought to the ship and incubated at vent temperature (23°C), higher rates of around 30 nmol C L^{-1} hr^{-1} were measured. These rates were as high as those measured for incubations at atmospheric pressure and 23°C to which 1 mM thiosulfate had been added.

These results point to the presence of pressure-adapted microbes, but equally important, if not more important, is the fact that, because they are likely to have a very strong effect on microbial activity, no outgassing of relevant chemical species (e.g., H_2, H_2S, CO_2) and no other changes in fluid geochemistry (e.g., pH) occurred during sample retrieval. However, significant time elapsed between taking the sample and the addition of the tracer onboard the ship, likely resulting in underestimation of the rates as available substrates in the chamber were being consumed, beginning at the time of sample collection. Also, these incubations were all carried out over relatively long time periods, often exceeding 24 hours. These long incubation times introduce additional biases, as cell numbers increase and community changes are likely to occur during these periods, as recently demonstrated by Perner et al. (2010). All of these analyses indicate that, ideally, incubations need to be carried out at both in situ pressure and in situ vent temperature over reasonably short time periods to obtain more realistic values that can be used to assess the productivity of these systems.

However, measuring rates of carbon fixation in situ at deep-sea hydrothermal vents represents a formidable task for a variety of reasons, ranging from the difficulty of designing instruments to function at high pressure and in the presence of reducing chemicals, to the question of under which condition(s) carbon production should be measured. In fact, in contrast to measuring primary production in the photic zone, deep-sea vents encompass a large range of environmental conditions (e.g., cold-hot, aerobic-anaerobic) that occur over relatively small spatial scales and that must be considered. In any case, developments are underway to enable these kinds of measurements and to couple them with techniques that can address which organisms are actually performing the reactions and which genes and pathways are expressed. Particularly noteworthy in this regard are employing HISH-SIMS to identify active microbes and using functional gene arrays, like the GeoChip, to assess the presence (Wang et al., 2009) and monitor expression of key genes. However, with "omic" techniques becoming ever more sensitive and requiring less biomass, applying these techniques to small volume samples approaches the realm of possibility. A fascinating approach would be to detect actively metabolizing cells and to subsequently sort them and sequence their genomes.

CHEMOAUTOTROPHIC MICROBIAL BIOFILMS

Early studies of the ecology of deep-sea hydrothermal vents included observations of white microbial growth on the basalt in proximity to diffuse-flow vents, as well as descriptions of microbial attachment to animate and inanimate surfaces (Lonsdale, 1977; Jannasch and Wirsen, 1979, 1981; Jannasch and Taylor, 1984; Jannasch, 1985). More recently, several studies addressed the importance of microbial attachment and biofilm formation at both focused- and diffuse-flow vents (reviewed in Schrenk et al., 2008). Yet, remarkably little information is currently available on the mechanisms underlying the establishment and development of microbial biofilms at deep-sea vents. Chemoautotrophic microorganisms are the first to attach to solid substrates (at and below the seafloor) at new vents where they form extensive biofilms that may play a critical role in "conditioning" the environment for metazoan settlement (Taylor et al., 1999; López-García et al., 2003; Alain et al., 2004). Based on current knowledge, the microorganisms that colonize deep-sea vents are expected to be able to adhere to solid substrates (to better access nutrients in these highly turbulent environments), to be metabolically versatile (to take advantage of the dynamic redox gradients typical of these environments) and, possibly, to use detoxification mechanisms that allow them to survive in an environment where concentrations of many heavy metals (e.g., Cu, Zn, Fe, Cd, Pb, Hg) and other chemicals frequently exceed levels normally considered toxic to organisms (Figure 2).

However, the relevance of biofilm-forming vs. free-swimming microbes has not been investigated. It is reasonable to hypothesize that attachment and formation of biofilms on native basalt in close proximity to vent emissions provides microorganisms with continuous access to reduced compounds used in energy-yielding processes (e.g., hydrogen sulfide in hydrothermal fluids; McCollom and Shock, 1997). In contrast, free-swimming bacteria contained in the fluids are advected into the surrounding water column, and thus away from their energy sources. In mildly oxidizing hydrothermal fluids, sulfur compounds are known to bind heavy metals, in turn reducing their bioavailability and toxicity (Von Damm, 1990, 1995). However, heavy metals may be mobilized and/or detoxified by microbial activities mediated by genetically encoded functions (Vetriani et al., 2005) and, possibly, by biosorption to the biofilm matrix (with implications for biomineralization processes). Overall, such detoxification mechanisms are consistent with the elevated concentrations of heavy metals (e.g., Hg up to 88.7 ng L^{-1} at the Tica site on the EPR) and high relative abundances of mercury-resistant bacteria that we measured in diffuse flow fluids at 9°N on the EPR (Crespo-Medina et al., 2009a).

The recent availability of genome sequences from chemoautotrophic vent organisms is revealing interesting functions related to the establishment of biofilms. For instance, we know that several genes involved in the biosynthesis of exopolysaccharides and in quorum sensing mechanisms are encoded in the genomes of four sulfur metabolizing *Epsilonproteobacteria* known to colonize vent environments: *Caminibacter mediatlanticus* (Govannelli et al., 2011), *Nautilia profundicola* (Campbell et al., 2009), *Sulfurovum* sp., and *Nitratiruptor* sp. (Nakagawa et al., 2007). Author Vetriani, Ileana Pérez-Rodriguez, and Jessica Ricci have obtained preliminary data demonstrating that pure cultures of *C. mediatlanticus* and *Sulfurovum* sp. express quorum-sensing-related genes during growth, and that these genes are also expressed in situ by the biofilm communities. These findings are in line with a study that showed a link among biofilm formation, exopolysaccharide synthesis, and quorum sensing in a hyperthermophilic bacterium (Johnson et al., 2005). We are currently applying transcriptomic and proteomic techniques in conjunction with geochemical measurements, thermodynamic modeling, and carefully designed experiments in order to shed light on the hypothesized processes described above.

FUTURE DIRECTION AND CHALLENGES— A PATH FORWARD

To achieve a better understanding of the fluxes of energy and matter at deep-sea vents and to characterize the microbially catalyzed processes underlying these transformations requires an integrated approach that couples an assessment of taxonomic diversity using cultivation-dependent and -independent approaches with methodologies addressing genetic diversity, including (1) metagenomics (genetic potential and diversity of a community), (2) single-cell genomics (genetic potential and diversity of uncultured single cells), (3) metatranscriptomics, and (4) metaproteomics (identification and function of active community members; realized potential of the community). To assess the functional component, these approaches can be combined with (1) measurement of in situ rates of chemoautotrophic productivity, (2) geochemical characterization of microbial habitats, and (3) shipboard incubations under

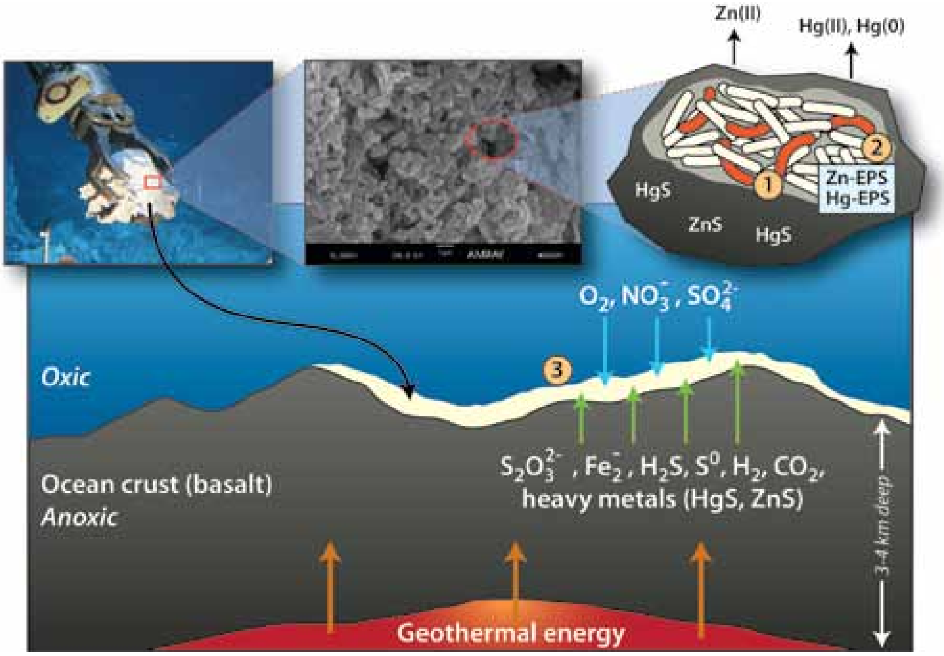

Figure 2. Diagram of a hypothetical basalt-colonizing microbial community at the oxic/anoxic interface of a diffuse flow vent. (1) A low-complexity community of chemosynthetic (straight, abundant) and heterotrophic (curved and red, sparse) bacteria attach to the basalt and establish a biofilm. When chemosynthetic bacteria oxidize sulfur species, they mobilize heavy metals, increasing their toxicity. (2) Bacteria in biofilms produce EPSs (extracellular polymeric substances), which sequester heavy metals, reducing their toxicity. Heterotrophic bacteria actively detoxify heavy metals. (3) Colonizing bacteria are metabolically versatile and can use multiple energy sources and terminal electron acceptors. Figure not drawn to scale.

simulated in situ conditions (hypothesis testing under controlled conditions). The integration of laboratory and field experiments in combination with a suite of cutting-edge analytical techniques, including analyses at the single-cell level, in a hypothesis-driven study of microbial processes will create unprecedented opportunities that will lead to a better understanding of the functioning of deep-sea vent microbial communities and the constraints regulating interactions between microbes and their abiotic and biotic environments (Figure 3).

A major challenge will be integrating and synthesizing the massive amount of generated data to reconstruct and simulate the metabolic network of the microbial communities contained in the vent fluids and to gather a comprehensive understanding of the system. However, methods have been developed for the integrated study of individual genomes that can be adapted to the whole community (e.g., Covert et al., 2001, 2004; Stolyar et al., 2007; Zhang et al., 2009; Feist et al., 2009). In such an approach, the community-specific "-omics" data can be linked to geochemical and physicochemical data to identify the pathways responsible for the adaptation of microbial communities to specific environmental conditions. In addition, metabolic simulation can be combined with environmental data to mimic the carbon and energy production of single cells, with the simulation possibly extending to the community level toward understanding and predicting carbon and energy transfers among different organisms. Flux balance analysis (Raman and Chandra, 2009) can be further adapted to simulate biomass generation and reaction fluxes at the scale of community-level metabolic networks, and the simulation results can be compared with experimental data to help identify knowledge gaps and improve our understanding of the key pathways in a number of processes, including biofilm formation.

It is an exciting time in this field because we are now in a position to connect the early days of deep-sea vent research, when there was greater focus on rate measurements, with the present focus on assessing microbial diversity. Now is the time to combine the presently available powerful "omic" and single-cell tools with thermodynamic modeling, experimental approaches, and new in situ instrumentation to measure rates and concentrations. The overarching goal is to bring our understanding of these truly fascinating ecosystems to a new level and to place them in a quantitative framework and thus a larger global context.

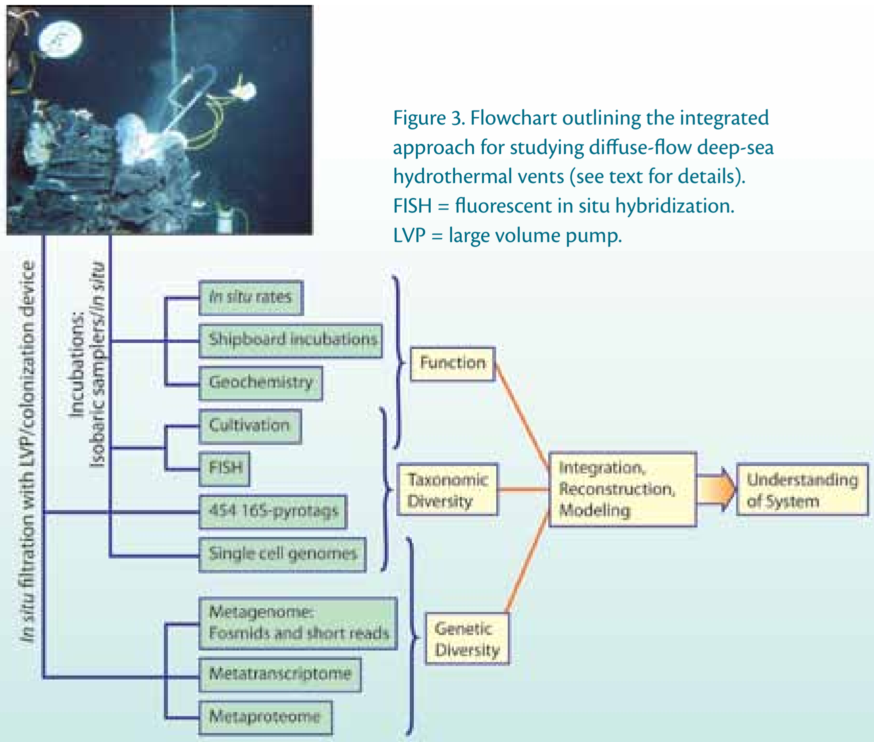

Figure 3. Flowchart outlining the integrated approach for studying diffuse-flow deep-sea hydrothermal vents (see text for details). FISH = fluorescent in situ hybridization. LVP = large volume pump.

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REFERENCES

Alain, K., N. Callac, M. Guegan, F. Lesongeur, P. Crassous, M.A. Cambon-Bonavita, J. Querellou, and D. Prieur. 2009. *Nautilia abyssi* sp. nov., a thermophilic, chemolithoautotrophic, sulfur-reducing bacterium isolated from an East Pacific Rise hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* 59:1,310–1,315, http://dx.doi.org/10.1099/ijs.0.005454-0.

Alain, K., A. Postec, E. Grinsard, F. Lesongeur, D. Prieur, and A. Godfroy. 2010. *Thermodesulfatator atlanticus* sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a Mid-Atlantic Ridge hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* 60:33–38, http://dx.doi.org/10.1099/ijs.0.009449-0.

Alain, K., J. Querellou, F. Lesongeur, P. Pignet, P. Crassous, G. Raguenes, V. Cueff, and M.A. Cambon-Bonavita. 2002. *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* 52:1,317–1,323, http://dx.doi.org/10.1099/ijs.0.02195-0.

Alain, K., S. Rolland, P. Crassous, F. Lesongeur, M. Zbinden, C. Le Gall, A. Godfroy, A. Page, S.K. Juniper, M.A. Cambon-Bonavita, and others. 2003. *Desulfurobacterium crinifex* sp. nov., a novel thermophilic, pinkish-streamer forming, chemolithoautotrophic bacterium isolated from a Juan de Fuca Ridge hydrothermal vent and amendment of the genus *Desulfurobacterium*. *Extremophiles* 7:361–370.

Alain, K., M. Zbinden, N. Le Bris, F. Lesongeur, J. Querellou, F. Gaill, and M.A. Cambon-Bonavita. 2004. Early steps in microbial colonization processes at deep-sea hydrothermal vents. *Environmental Microbiology* 6:227–241, http://dx.doi.org/10.1111/j.1462-2920.2003.00557.x.

Amend, J.P., and E.L. Shock. 2001. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and bacteria. *FEMS Microbiology Review* 25:175–243, http://dx.doi.org/10.1111/j.1574-6976.2001.tb00576.x.

Amend, J.P., T.M. McCollom, M. Hentscher, and W. Bach. 2011. Catabolic and anabolic energy for chemolithoautotrophs in deep-sea hydrothermal systems hosted in different rock types. *Geochimica et Cosmochimica Acta* 75:5,736–5,748, http://dx.doi.org/10.1016/j.gca.2011.07.041.

Bach, W., K.J. Edwards, J.M. Hayes, J.A. Huber, S.M. Sievert, and M.L. Sogin. 2006. Energy in the dark: Fuel for life in the deep ocean and beyond. *Eos, Transactions, American Geophysical Union* 87(7):73, http://dx.doi.org/10.1029/2006EO070002.

Blöchl, E., R. Rachel, S. Burggraf, D. Hafenbradl, H.W. Jannasch, and K.O. Stetter. 1997. *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 degrees C. *Extremophiles* 1:14–21, http://dx.doi.org/10.1007/s007920050010.

Brazelton, W.J., and J.A. Baross. 2009. Abundant transposases encoded by the metagenome of a hydrothermal chimney biofilm. *ISME Journal* 3:1,420–1,424, http://dx.doi.org/10.1038/ismej.2009.79.

Brazelton, W.J., and J.A. Baross. 2010. Metagenomic comparison of two *Thiomicrospira* lineages inhabiting contrasting deep-sea hydrothermal environments. *PLoS One* 5:8, http://dx.doi.org/10.1371/journal.pone.0013530.

Butterfield, D.A., K.K. Roe, M.D. Lilley, J.A. Huber, J.A. Baross, R.W. Embley, and G.J. Massoth. 2004. Mixing, reaction and microbial activity in the sub-seafloor revealed by temporal and spatial variation in diffuse flow vents at Axial Volcano. Pp. 269–289 in *Subseafloor Biosphere at Mid-Oceanic Ridges*. W.S.D. Wilcock, E.F. DeLong, D.S. Kelley, J.A. Baross, and S.C. Cary, eds, Geophysical Monograph Series, vol. 144, American Geophysical Union, Washington, DC.

Campbell, B.C., A.S. Engel, M.L. Porter, and K. Takai. 2006. The versatile ε–proteobacteria: Key players in sulphidic habitats. *Nature Reviews Microbiology* 4:458–468, http://dx.doi.org/10.1038/nrmicro1414.

Campbell, B.J., C. Jeanthon, J.E. Kostka, G.W. Luther III, and S.C. Cary. 2001. Growth and phylogenetic properties of novel bacteria belonging to the epsilon subdivision of the proteobacteria enriched from *Alvinella pompejana* and deep-sea hydrothermal vents. *Applied and Environmental Microbiology* 67:4,566–4,572, http://dx.doi.org/10.1128/AEM.67.10.4566-4572.2001.

Campbell, B.J., J.L. Smith, T.E. Hanson, M.G. Klotz, L.Y. Stein, C.K. Lee, D. Wu, J.M. Robinson, H.M. Khouri, J.A. Eisen, and S.C. Cary. 2009. Adaptations to submarine hydrothermal environments exemplified by the genome of *Nautilia profundicola*. *PLoS Genetics* 5:e1000362, http://dx.doi.org/10.1371/journal.pgen.1000362.

Corliss, J.B., J. Dymond, L.I. Gordon, J.M. Edmond, R.P. von Herzen, R.D. Ballard, K. Green, D. Williams, A. Bainbridge, K. Crane, and T.H. von Andel. 1979. Submarine thermal springs on the Galapagos Rift. *Science* 203:1,073–1,083, http://dx.doi.org/10.1126/science.203.4385.1073.

Covert, M.W., E.M. Knight, J.L. Reed, M.J. Herrgard, and B.O. Palsson. 2004. Integrating high-throughput and computational data elucidates bacterial networks. *Nature* 429:92–96, http://dx.doi.org/10.1038/nature02456.

Covert, M.W., C.H. Schilling, I. Famili, J.S. Edwards, I.I. Goryanin, E. Selkov, and B.O. Palsson. 2001. Metabolic modeling of microbial strains in silico. *Trends in Biochemical Sciences* 26:179–186, http://dx.doi.org/10.1016/S0968-0004(00)01754-0.

Crespo-Medina, M., A.D. Chatziefthimiou, N.S. Bloom, G.W. Luther, J.R. Reinfelder, C. Vetriani, and T. Barkay. 2009a. Adaptation of chemosynthetic microorganisms to elevated mercury concentrations in deep-sea hydrothermal vents. *Limnology and Oceanography* 54:41–49, http://dx.doi.org/10.4319/lo.2009.54.1.0041.

Crespo-Medina, M., A. Chatziefthimiou, R. Cruz-Matos, I. Pérez-Rodriguez, T. Barkay, R.A. Lutz, V. Starovoytov, and C. Vetriani. 2009b. *Salinisphaera hydrothermalis* sp. nov., a mesophilic, halotolerant, facultatively autotrophic, thiosulfate-oxidizing gammaproteobacterium from deep-sea hydrothermal vents, and emended description of the genus *Salinisphaera*. *International Journal of Systematic and Evolutionary Microbiology* 59:1,497–1,503, http://dx.doi.org/10.1099/ijs.0.005058-0.

DeLong, E.F. 2009. The microbial ocean from genomes to biomes. *Nature* 459:200–206, http://dx.doi.org/10.1038/nature08059.

DeLong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N.-U. Frigaard, A. Martinez, M.B. Sulivan, R. Edwards, B. Rodriguez Brito, and others. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311:496–503, http://dx.doi.org/10.1126/science.1120250.

Durand, P., A.-L. Reysenbach, D. Prieur, and N.R. Pace. 1993. Isolation and characterization of *Thiobacillus hydrothermalis* sp. nov., a mesophilic obligately chemolithotrophic bacterium isolated from a deep-sea hydrothermal vent in the Fiji Basin. *Archives of Microbiology* 159:39–44.

Eloe, E.A., D.W. Fadrosh, M. Novotny, L.Z. Allen, M. Kim, M.J. Lombardo, J. Yee-Greenbaum, S. Yooseph, E.E. Allen, R. Lasken, and others. 2011. Going deeper: Metagenome of a hadopelagic microbial community. *PLoS One* 6(5):e20388, http://dx.doi.org/10.1371/journal.pone.0020388.

Feist, A.M., M.J. Herrgard, I. Thiele, J.L. Reed, and B.O. Palsson. 2009. Reconstruction of biochemical networks in microorganisms. *Nature Reviews Microbiology* 7:129–143, http://dx.doi.org/10.1038/nrmicro1949.

Flores, G.E., J.H. Campbell, J.D. Kirshtein, J. Meneghin, M. Podar, J.I. Steinberg, J.S. Seewald, M.K. Tivey, M.A. Voytek, Z.K. Yang, and A.L. Reysenbach. Microbial community structure of hydrothermal deposits from geochemically different vent fields along the Mid-Atlantic Ridge. *Environmental Microbiology* 13:2,158–2,171, http://dx.doi.org/10.1111/j.1462-2920.2011.02463.x.

Foustoukos, D.I., J.L. Houghton, W.E. Seyfried Jr., S.M. Sievert, and G.D. Cody. 2011. Kinetics of H_2-O_2-H_2O redox equilibria and formation of metastable H_2O_2 under low temperature hydrothermal conditions. *Geochemica et Cosmochimica Acta* 75:1,594–1,607, http://dx.doi.org/10.1016/j.gca.2010.12.020.

Gardebrecht, A., S. Markert, S.M. Sievert, H. Felbeck, A.Thürmer, D. Albrecht, A. Wollherr, J. Kabisch, N. Le Bris, R. Lehmann, and others. 2011. Physiological homogeneity among the endosymbionts of *Riftia pachyptila* and *Tevnia jerichonana* revealed by proteogenomics. *The ISME Journal*, http://dx.doi.org/10.1038/ismej.2011.137.

Gilbert, J.A., and C.L. Dupont. 2011. Microbial metagenomics: Beyond the genome. *Annual Review in Marine Science* 3:347–371, http://dx.doi.org/10.1146/annurev-marine-120709-142811.

Giovannelli, D., S. Ferriera, J. Johnson, S. Kravitz, I. Pérez-Rodriguez, J. Ricci, C. O'Brien, J.W. Voordeckers, E. Bini, and C. Vetriani. 2011. Draft genome sequence of *Caminibacter mediatlanticus* strain TB-2^T, an epsiloproteobacterium isolated from a deep-sea hydrothermal vent. *Standards in Genomic Science* 5:135–143, http://dx.doi.org/10.4056/sigs.2094859.

Götz, D., A. Banta, T.J. Beveridge, A.I. Rushdi, B.R.T. Simoneit, and A. Reysenbach. 2002. *Persephonella marina* gen. nov., sp nov. and *Persephonella guaymasensis* sp nov., two novel, thermophilic, hydrogen- oxidizing microaerophiles from deep-sea hydrothermal vents. *International Journal of Systematic and Evolutionary Microbiology* 52:1,349–1,359, http://dx.doi.org/10.1099/ijs.0.02126-0.

Grzymski, J.J., A.E. Murray, B.J. Campbell, M. Kaplarevic, G.R. Gao, C. Lee, R. Daniel, A. Ghadiri, R.A. Feldman, and S.C. Cary. 2008. Metagenome analysis of an extreme microbial symbiosis reveals eurythermal adaptation and metabolic flexibility. *Proceedings of the National Academy of Sciences of the United States of America* 105:17,516–17,521, http://dx.doi.org/10.1073/pnas.0802782105.

Holden, J.F., J.A. Breier, K.L. Rogers, M.D. Schulte, and B.M. Toner. 2012. Biogeochemical processes at hydrothermal vents: Microbes and minerals, bioenergetics, and carbon fluxes. *Oceanography* 25(1):196–208, http://dx.doi.org/10.5670/oceanog.2012.18.

Houghton, J.L., and W.E. Seyfried Jr. 2010. An experimental and theoretical approach to determining linkages between geochemical variability and microbial biodiversity in seafloor hydrothermal chimneys. *Geobiology* 8:457–470, http://dx.doi.org/10.1111/j.1472-4669.2010.00255.x.

Houghton, J.L., W.E. Seyfried Jr., A.B. Banta, and A.-L. Reysenbach. 2007. Continuous enrichment culturing of thermophiles under sulfate and nitrate-reducing conditions and at deep-sea hydrostatic pressures. *Extremophiles* 11:371–382, http://dx.doi.org/10.1007/s00792-006-0049-7.

Huber, H., S. Burggraf, T. Mayer, I. Wyschkony, R. Rachel, and K.O. Stetter. 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* 6:2,093–2,100.

Huber, J.A., D.A. Butterfield, and J.A. Baross. 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiology Ecology* 43:393–409, http://dx.doi.org/10.1111/j.1574-6941.2003.tb01080.x.

Huber, H., H.W. Jannasch, R. Rachel, T. Fuchs, and K.O. Stetter. 1997. *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers. *Systematic and Applied Microbio*logy 20:374–380, http://dx.doi.org/10.1016/S0723-2020(97)80005-7.

Huber, J.A., D.B. Mark Welch, H.G. Morrison, S.M. Huse, P.R. Neal, D.A. Butterfield, and M.L. Sogin. 2007. Microbial population structures in the deep marine biosphere. *Science* 318:97–100, http://dx.doi.org/10.1126/science.1146689.

Hügler, M., and S.M. Sievert. 2011. Beyond the Calvin Cycle: Autotrophic carbon fixation in the ocean. *Annual Review of Marine Science* 3:261–289, http://dx.doi.org/10.1146/annurev-marine-120709-142712.

Hügler, M., J.M. Petersen, N. Dubilier, J.F. Imhoff, and S.M. Sievert. 2011. Pathways of carbon and energy metabolism of the epibiotic community associated with the deep-sea hydrothermal vent shrimp *Rimicaris exoculata*. *PLoS One* 6(1):e16018, http://dx.doi.org/10.1371/journal.pone.0016018.

Inagaki, F., K. Takai, H. Kobayashi, K.H. Nealson, and K. Horikoshi. 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* 53:1,801–1,805, http://dx.doi.org/10.1099/ijs.0.02682-0.

Inagaki, F., K. Takai, K.H. Nealson, and K. Horikoshi. 2004. *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the *ε-Proteobacteria* isolated from Okinawa Trough hydrothermal sediments. *International Journal of Systematic and Evolutionary Microbiology* 54:1,477–1,482, http://dx.doi.org/10.1099/ijs.0.03042-0.

Jannasch, H.W. 1985. The chemosynthetic support of life and the microbial diversity at deep-sea hydrothermal vents. *Proceedings of the Royal Society of London B* 225:277–297, http://dx.doi.org/10.1098/rspb.1985.0062.

Jannasch, H.W. 1995. Microbial interactions with hydrothermal fluids. Pp. 273–296 in *Seafloor Hydrothermal Systems*. S.E. Humphris, R.A. Zierenberg, L.S. Mullineaux, and R.E. Thomson, eds, Geophysical Monograph Series, vol. 91, American Geophysical Union, Washington, DC.

Jannasch, H.W., and M.J. Mottl. 1985. Geomicrobiology of deep-sea hydrothermal vents. *Science* 229:717–725, http://dx.doi.org/10.1126/science.229.4715.717.

Jannasch, H.W., and C.D. Taylor. 1984. Deep-sea microbiology. *Annual Review of Microbiology* 38:487–514, http://dx.doi.org/10.1146/annurev.mi.38.100184.002415.

Jannasch, H.W., and C.O. Wirsen. 1979. Chemosynthetic primary production at East Pacific sea floor spreading centers. *Bioscience* 29:592–598.

Jannasch, H.W., and C.O. Wirsen. 1981. Morphological survey of microbial mats near deep-sea thermal vents. *Applied and Environmental Microbiology* 41:528–538.

Jannasch, H.W., C.O. Wirsen, D.C. Nelson, and L.A. Robertson. 1985. *Thiomicrospira crunogena* sp. nov., a colorless sulfur-oxidizing bacterium from a deep-sea hydrothermal vent. *International Journal of Systematic Bacteriology* 35:422–424, http://dx.doi.org/10.1099/00207713-35-4-422.

Jeanthon, C., S. L'Haridon, V. Cueff, A. Banta, A.L. Reysenbach, and D. Prieur. 2002. *Thermodesulfobacterium hydrogeniphilum* sp nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a deep-sea hydrothermal vent at Guaymas Basin, and emendation of the genus *Thermodesulfobacterium*. *International Journal of Systematic and Evolutionary Microbiology* 52:765–772, http://dx.doi.org/10.1099/ijs.0.02025-0.

Johnson, M.R., C.I. Montero, S.B. Conners, K.R. Shockley, S.L. Bridger, and R.M. Kelly. 2005. Population density-dependent regulation of exopolysaccharide formation in the hyperthermophilic bacterium *Thermotoga maritima*. *Molecular Microbiology* 55:664–674, http://dx.doi.org/10.1111/j.1365-2958.2004.04419.x.

Karl, D.M. 1995. Ecology of free-living, hydrothermal vent microbial communities. Pp. 35–124 in *Microbiology of Deep-Sea Hydrothermal Vents*. D.M. Karl, ed., CRC Press, Boca Raton, FL.

Kashefi, K., and D.R. Lovley. 2003. Extending the upper temperature limit for life. *Science* 301:934, http://dx.doi.org/10.1126/science.1086823.

Kashefi, K., J.M. Tor, D.E. Holmes, C.V. Gaw Van Praagh, A.L. Reysenbach, and D.R. Lovley. 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, http://dx.doi.org/10.1099/ijs.0.01953-0.

Kelley, D.S., J.A. Baross, and J.R. Delaney. 2002. Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annual Review of Earth and Planetary Sciences* 30:385–491, http://dx.doi.org/10.1146/annurev.earth.30.091201.141331.

Kurr, M., R. Huber, H. Konig, H.W. Jannasch, H. Fricke, A. Trincone, J.K. Kristjansson, and K.O. Stetter. 1991. *Methanopyrus-Kandleri*, Gen and Sp-Nov represents a novel group of hyperthermophilic methanogens, growing at 110°C. *Archives of Microbiology* 156:239–247, http://dx.doi.org/10.1007/BF00262992.

L'Haridon, S., V. Cilia, P. Messner, G. Raguenes, A. Gambacorta, U.B. Sleytr, D. Prieur, and C. Jeanthon. 1998. *Desulfurobacterium thermolithotrophum* gen. nov., sp. nov., a novel autotrophic, sulphur-reducing bacterium isolated from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* 48:701–711, http://dx.doi.org/10.1099/00207713-48-3-701.

L'Haridon, S., A.L. Reysenbach, A. Banta, P. Messner, P. Schumann, E. Stackebrandt, and C. Jeanthon. 2003. *Methanocaldococcus indicus* sp. nov., a novel hyperthermophilic methanogen isolated from the Central Indian Ridge. *International Journal of Systematic and Evolutionary Microbiology* 53:1,931–1,935, http://dx.doi.org/10.1099/ijs.0.02700-0.

L'Haridon, S., A.L. Reysenbach, B.J. Tindall, P. Schonheit, A. Banta, U. Johnsen, P. Schumann, A. Gambacorta, E. Stackebrandt, and C. Jeanthon. 2006. *Desulfurobacterium atlanticum* sp. nov., *Desulfurobacterium pacificum* sp. nov. and *Thermovibrio guaymasensis* sp. nov., three thermophilic members of the *Desulfurobacteriaceae* fam. nov., a deep branching lineage within the *Bacteria*. *International Journal of Systematic and Evolutionary Microbiology* 56:2,843–2,852, http://dx.doi.org/10.1099/ijs.0.63994-0.

Lonsdale, P. 1977. Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep Sea Research* 24:857–858, http://dx.doi.org/10.1016/0146-6291(77)90478-7.

López-García, P., S. Duperron, P. Philippot, J. Foriel, J. Susini, and D. Moreira. 2003. Bacterial diversity in hydrothermal sediment and epsilonproteobacterial dominance in experimental microcolonizers at the Mid-Atlantic Ridge. *Environmental Microbiology* 5:961–976, http://dx.doi.org/10.1046/j.1462-2920.2003.00495.x.

Markert, S., C. Arndt, H. Felbeck, D. Becher, S.M. Sievert, M. Hügler, D. Albrecht, J. Robidart, S. Bench, R.A. Feldman, and others. 2007. Physiological proteomics of the uncultured endosymbiont of *Riftia pachyptila*. *Science* 315:247–250, http://dx.doi.org/10.1126/science.1132913.

Markert, S., A. Gardebrecht, H. Felbeck, S.M. Sievert, J. Klose, D. Becher, D. Albrecht, A. Thurmer, R. Daniel, M. Kleiner, and others. 2011. Status quo in physiological proteomics of the uncultured *Riftia pachyptila* endosymbiont. *Proteomics* 11:3,106–3,117, http://dx.doi.org/10.1002/pmic.201100059.

McCarren, J., J.W. Becker, D.J. Repeta, Y.M. Shi, C.R. Young, R.R. Malmstrom, S.W. Chisholm, and E.F. DeLong. 2010. Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proceedings of the National Academy of Sciences of the United States of America* 107:16,420–16,427, http://dx.doi.org/10.1073/pnas.1010732107.

McCollom, T.M., and E.L. Shock. 1997. Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems. *Geochimica et Cosmochimica Acta* 61:4,375–4,391, http://dx.doi.org/10.1016/S0016-7037(97)00241-X.

Miroshnichenko, M.L., S. L'Haridon, P. Schumann, S. Spring, E.A. Bonch-Osmolovskaya, C. Jeanthon, and E. Stackebrandt. 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, http://dx.doi.org/10.1099/ijs.0.02753-0.

Miroshnichenko, M.L., N.A. Kostrikina, S. L'Haridon, C. Jeanthon, H. Hippe, E. Stackebrandt, and E.A. Bonch-Osmolovskaya. 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* 52:1,299–1,304, http://dx.doi.org/10.1099/ijs.0.02139-0.

Miroshnichenko, M.L., A.I. Slobodkin, N.A. Kostrikina, S. L'Haridon, O. Nercessian, S. Spring, E. Stackebrandt, E.A. Bonch-Osmolovskaya, and C. Jeanthon. 2003. *Deferribacter abyssi* sp. nov., an anaerobic thermophile from deep-sea hydrothermal vents of the Mid-Atlantic Ridge. *International Journal of Systematic and Evolutionary Microbiology* 53:1,637–1,641, http://dx.doi.org/10.1099/ijs.0.02673-0.

Mori, K., K. Suzuki, T. Urabe, M. Sugihara, K. Tanaka, M. Hamada, and S. Hanada. 2011. *Thioprofundum hispidum* sp. nov., an obligately chemolithoautotrophic sulfur-oxidizing gammaproteobacterium isolated from the hydrothermal field on Suiyo Seamount, and proposal of *Thioalkalispiraceae* fam. nov. in the order *Chromatiales*. *International Journal of Systematic and Evolutionary Microbiology* 61:2,412–2,418, http://dx.doi.org/10.1099/ijs.0.026963-0.

Morris, R.M., B.L. Nunn, C. Frazar, D.R. Goodlett, Y.S. Ting, and G. Rocap. 2010. Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction. *The ISME Journal* 4:673–685, http://dx.doi.org/10.1038/ismej.2010.4.

Moussard, H., S. L'Haridon, B.J. Tindall, A. Banta, P. Schumann, E. Stackebrandt, A.L. Reysenbach, and C. Jeanthon. 2004. *Thermodesulfatator indicus* gen. nov., sp. nov., a novel thermophilic chemolithoautotrophic sulfate-reducing bacterium isolated from the Central Indian Ridge. *International Journal of Systematic and Evolutionary Microbiology* 54:227–233, http://dx.doi.org/10.1099/ijs.0.02669-0.

Musat, N., H. Halm, B. Winterholler, P. Hoppe, S. Peduzzi, F. Hillion, F. Horreard, R. Amann, B.B. Jørgensen, and M.M.M. Kuypers. 2008. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 105:17,861–17,866, http://dx.doi.org/10.1073/pnas.0809329105.

Nakagawa, S., and K. Takai. 2008. Deep-sea vent chemoautotrophs: Diversity, biochemistry, and ecological significance. *FEMS Microbiology Ecology* 65:1–14, http://dx.doi.org/10.1111/j.1574-6941.2008.00502.x.

Nakagawa, S., F. Inagaki, K. Takai, K. Horikoshi, and Y. Sako. 2005a. *Thioreductor micantisoli* gen. nov., sp. nov., a novel mesophilic, sulfur-reducing chemolithoautotroph within the *ε-Proteobacteria* isolated from hydrothermal sediments in the Mid-Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* 55:599–605, http://dx.doi.org/10.1099/ijs.0.63351-0.

Nakagawa, S., K. Takai, K. Horikoshi, and Y. Sako. 2003. *Persephonella hydrogeniphila* sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium from a deep-sea hydrothermal vent chimney. *International Journal of Systematic and Evolutionary Microbiology* 53:863–869, http://dx.doi.org/10.1099/ijs.0.02505-0.

Nakagawa, S., K. Takai, F. Inagaki, K. Horikoshi, and Y. Sako. 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, http://dx.doi.org/10.1099/ijs.0.63480-0.

Nakagawa, S., Y. Takaki, S. Shimamura, A.L. Reysenbach, K. Takai, and K. Horikoshi. 2007. Deep-sea vent ε-proteobacterial genomes provide insights into emergence of pathogens. *Proceedings of the National*

Academy of Sciences of the United States of America 104:12,146–12,150, http://dx.doi.org/10.1073/pnas.0700687104.

Nakamura, R., T. Takashima, S. Kato, K. Takai, M. Yamamoto, and K. Hashimoto. 2010. Electrical current generation across a black smoker chimney. *Angewandte Chemie* International Edition 49:7,692–7,694, http://dx.doi.org/10.1002/anie.201003311.

Nunoura, T., M. Miyazaki, Y. Suzuki, K. Takai, and K. Horikoshi. 2008a. *Hydrogenivirga okinawensis* sp. nov., a thermophilic sulfur-oxidizing chemolithoautotroph isolated from a deep-sea hydrothermal field, Southern Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* 58:676–681, http://dx.doi.org/10.1099/ijs.0.64615-0.

Nunoura, T., H. Oida, M. Miyazaki, and Y. Suzuki. 2008b. *Thermosulfidibacter takaii* gen. nov., sp. nov., a thermophilic, hydrogen-oxidizing, sulfur-reducing chemolithoautotroph isolated from a deep-sea hydrothermal field in the Southern Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* 58:659–665, http://dx.doi.org/10.1099/ijs.0.65349-0.

Opatkiewicz, A.D., D.A. Butterfield, and J.A. Baross. 2009. Individual hydrothermal vents at Axial Seamount harbor distinct subseafloor microbial communities. *FEMS Microbiology Ecology* 70:413–424, http://dx.doi.org/10.1111/j.1574-6941.2009.00747.x.

Perner, M., J.M. Petersen, F. Zielinski, H.H. Gennerich, and R. Seifert. 2010. Geochemical constraints on the diversity and activity of H_2-oxidizing microorganisms in diffuse hydrothermal fluids from a basalt- and an ultramafic-hosted vent. *FEMS Microbiology Ecology* 74:55–71, http://dx.doi.org/10.1111/j.1574-6941.2010.00940.x.

Pérez-Rodriguez, I., A. Grosche, L. Massenburg, V. Starovoytov, R.A. Lutz, and C. Vetriani. In press. *Phorcysia thermohydrogeniphila* gen. nov. sp. nov., a thermophilic, chemolithoautotrophic, nitrate-ammonifying bacterium from a deep-sea hydrothermal vent on the East Pacific Rise. *International Journal of Systematic and Evolutionary Microbiology*.

Pérez-Rodriguez, I., J. Ricci, J.W. Voordeckers, V. Starovoytov, and C. Vetriani. 2009. *Nautilia nitratireducens* sp. nov., a thermophilic, anaerobic, chemosynthetic, nitrate-ammonifying bacterium isolated from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* 60:1,182–1,186, http://dx.doi.org/10.1099/ijs.0.013904-0.

Petersen, J.M., F.U. Zielinski, T. Pape, R. Seifert, C. Moraru, R. Amann, S. Hourdez, P.R. Girguis, S.D. Wankel, V. Barbe, and others. 2011. Hydrogen is an energy source for hydrothermal vent symbioses. *Nature* 476:176–180, http://dx.doi.org/10.1038/nature10325.

Raman, K., and N. Chandra. 2009. Flux balance analysis of biological systems: Applications and challenges. *Briefings in Bioinformatics* 10:435–449, http://dx.doi.org/10.1093/bib/bbp011.

Reysenbach, A.-L., and E. Shock. 2002. Merging genomes with geochemistry in hydrothermal ecosystems. *Science* 296:1,077–1,082, http://dx.doi.org/10.1126/science.1072483.

Robidart, J.C., A. Roque, P.F. Song, and P.R. Girguis. 2011. Linking hydrothermal geochemistry to organismal physiology: Physiological versatility in *Riftia pachyptila* from sedimented and basalt-hosted vents. *PLoS One* 6(7):e21692, http://dx.doi.org/10.1371/journal.pone.0021692.

Roussel, E.G., C. Konn, J.L. Charlou, J.P. Donval, Y. Fouquet, J. Querellou, D. Prieur, and M.A.C. Bonavita. 2011. Comparison of microbial communities associated with three Atlantic ultramafic hydrothermal systems. *FEMS Microbiology Ecology* 77:647–665, http://dx.doi.org/10.1111/j.1574-6941.2011.01161.x.

Schmidt, C., R. Vuillemin, C. Le Gall, F. Gaill, and N. Le Bris. 2008. Geochemical energy sources for microbial primary production in the environment of hydrothermal vent shrimps. *Marine Chemistry* 108:18–31, http://dx.doi.org/10.1016/j.marchem.2007.09.009.

Schrenk, M.O., J.F. Holden, and J.A. Baross. 2008. Magma to microbe networks in the context of sulfide hosted microbial ecosystems. Pp. 233–258 in *Magma to Microbe*. R.P. Lowell, J.F. Seewald, A. Metaxas, and M.R. Perfit, eds, Geophysical Monograph Series, vol. 178, American Geophysical Union, Washington, DC.

Schrenk, M.O., J.A. Huber, and K.J. Edwards. 2010. Microbial provinces in the subseafloor. *Annual Review of Marine Science* 2:279–304, http://dx.doi.org/10.1146/annurev-marine-120308-081000.

Shank, T.M., D.J. Fornari, K.L. Von Damm, M.D. Lilley, R.L. Haymon, and R.A. Lutz. 1998. Temporal and spatial patterns of biological community development at nascent deep-sea hydrothermal vents (9°50'N, East Pacific Rise). *Deep Sea Research Part II* 45:465–515, http://dx.doi.org/10.1016/S0967-0645(97)00089-1.

Shock, E.L., and M.E. Holland. 2004. Geochemical energy sources that support the subsurface biosphere. Pp. 153–165 in *Subseafloor Biosphere at Mid-Oceanic Ridges*. W.S.D. Wilcock, E.F. DeLong, D.S. Kelley, J.A. Baross, and S.C. Cary, eds, Geophysical Monograph Series, vol. 144, American Geophysical Union, Washington, DC.

Sievert, S.M., M. Hügler, C.O. Wirsen, and C.D. Taylor. 2007. Sulfur oxidation at deep-sea hydrothermal vents. Pp. 238–258 in *Microbial Sulfur Metabolism*. C. Dahl and C.G. Friedrich, eds, Springer, Berlin, Germany.

Slobodkina, G.B., T.V. Kolganova, N.A. Chernyh, J. Querellou, E.A. Bonch-Osmolovskaya, and A.I. Slobodkin. 2009. *Deferribacter autotrophicus* sp nov., an iron(III)-reducing bacterium from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology* 59:1,508–1,512, http://dx.doi.org/10.1099/ijs.0.006767-0.

Smith, J.L., B.J. Campbell, T.E. Hanson, C.L. Zhang, and S.C. Cary. 2008. *Nautilia profundicola* sp. nov., a thermophilic, sulfur-reducing epsilonproteobacterium from deep-sea hydrothermal vents. *International Journal of Systematic and Evolutionary Microbiology* 58:1,598–1,602, http://dx.doi.org/10.1099/ijs.0.65435-0.

Sowell, S.M., P.E. Abraham, M. Shah, N.C. Verberkmoes, D.P. Smith, D.F. Barofsky, and S.J. Giovannoni. 2010. Environmental proteomics of microbial plankton in a highly productive coastal upwelling system. *The ISME Journal* 5(5):856–865, http://dx.doi.org/10.1038/ismej.2010.168.

Steinsbu, B.O., I.H. Thorseth, S. Nakagawa, F. Inagaki, M.A. Lever, B. Engelen, L. Øvreås, and R.B. Pedersen. 2010. *Archaeoglobus sulfaticallidus* sp. nov., a novel thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids. *International Journal of Systematic and Evolutionary Microbiology* 60:2,745–2,752, http://dx.doi.org/10.1099/ijs.0.016105-0.

Stepanauskas, R., and M.E. Sieracki. 2007. Matching phylogeny and metabolism in the uncultured marine bacteria, one cell at a time. *Proceedings of the National Academy of Sciences of the United States of America* 104:9,052–9,057, http://dx.doi.org/10.1073/pnas.0700496104.

Stolyar, S., S. Van Dien, K.L. Hillesland, N. Pinel, T.J. Lie, J.A. Leigh, and D.A. Stahl. 2007. Metabolic modeling of a mutualistic microbial community. *Molecular Systems Biology* 3:92, http://dx.doi.org/10.1038/msb4100131.

Strohm, T.O., B. Griffin, W.G. Zumft, and B. Schink. 2007. Growth yields in bacterial denitrification and nitrate ammonification. *Applied and Environmental Microbiology* 73:1,420–1,424, http://dx.doi.org/10.1128/AEM.02508-06.

Swan, B.K., M. Martinez-Garcia, C.M. Preston, A. Sczyrba, T. Woyke, D. Lamy, T. Reinthaler, N.J. Poulton, E.D.P. Masland, M.L. Gomez, and others. 2011. Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. *Science* 333:1,296–1,300, http://dx.doi.org/10.1126/science.1203690.

Takai, K., and K. Nakamura. 2011. Archaeal diversity and community development in deep-sea hydrothermal vents. *Current Opinion in Microbiology* 14:282–291, http://dx.doi.org/10.1016/j.mib.2011.04.013.

Takai, K., H. Hirayama, T. Nakagawa, Y. Suzuki, K.H. Nealson, and K. Horikoshi. 2004a. *Thiomicrospira thermophila* sp. nov., a novel microaerobic, thermotolerant, sulfur-oxidizing chemolithomixotroph isolated from a deep-sea hydrothermal fumarole in the TOTO caldera, Mariana Arc, western Pacific. *International Journal of Systematic and Evolutionary Microbiology* 54:2,325–2,333, http://dx.doi.org/10.1099/ijs.0.63284-0.

Takai, K., H. Hirayama, T. Nakagawa, Y. Suzuki, K.H. Nealson, and K. Horikoshi. 2005. *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, http://dx.doi.org/10.1099/ijs.0.63330-0.

Takai, K., F. Inagaki, S. Nakagawa, H. Hirayama, T. Nunoura, Y. Sako, K.H. Nealson, and K. Horikoshi. 2003a. Isolation and phylogenetic diversity of members of previously uncultivated ε-proteobacteria in deep-sea hydrothermal fields. *FEMS Microbiology Letters* 218:167–174, http://dx.doi.org/10.1111/j.1574-6968.2003.tb11514.x.

Takai, K., A. Inoue, and K. Horikoshi. 2002. *Methanothermococcus okinawensis* sp. nov., a thermophilic, methane-producing archaeon isolated from a western Pacific deep-sea hydrothermal vent system. *International Journal of Systematic and Evolutionary Microbiology* 52:1,089–1,095, http://dx.doi.org/10.1099/ijs.0.02106-0.

Takai, K., M. Miyazaki, H. Hirayama, S. Nakagawa, J. Querellou, and A. Godfroy. 2009. Isolation and physiological characterization of two novel, piezophilic, thermophilic chemolithoautotrophs from a deep-sea hydrothermal vent chimney. *Environmental Microbiology* 11:1,983–1,997, http://dx.doi.org/10.1111/j.1462-2920.2009.01921.x.

Takai, K., S. Nakagawa, A.-L. Reysenbach, and J. Hoek. 2006a. Microbial ecology of mid-ocean ridges and back-arc basins. Pp. 185–213 in *Back-Arc Spreading Systems: Geological, Biological, Chemical and Physical Interactions*. D.M. Christie, C.R. Fisher, S.M. Lee, and S. Givens, eds, Geophysical Monograph Series, vol. 166, American Geophysical Union, Washington, DC.

Takai, K., S. Nakagawa, Y. Sako, and K. Horikoshi. 2003b. *Balnearium lithotrophicum* gen. nov., sp. nov., a novel thermophilic, strictly anaerobic, hydrogen-oxidizing chemolithoautotroph isolated from a black smoker chimney in the Suiyo Seamount hydrothermal system. *International Journal of Systematic and Evolutionary Microbiology* 53:1,947–1,954, http://dx.doi.org/10.1099/ijs.0.02773-0.

Takai, K., K. Nakamura, T. Toki, U. Tsunogai, M. Miyazaki, J. Miyazaki, H. Hirayama, S. Nakagawa, T. Nunoura, and K. Horikoshi. 2008. Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences of the United States of America* 105:10,949–10,954, http://dx.doi.org/10.1073/pnas.0712334105.

Takai, K., K.H. Nealson, and K. Horikoshi. 2004b. *Methanotorris formicicus* sp. nov., a novel extremely thermophilic, methane-producing archaeon isolated from a black smoker chimney in the Central Indian Ridge. *International Journal of Systematic and Evolutionary Microbiology* 54:1,095–1,100, http://dx.doi.org/10.1099/ijs.0.02887-0.

Takai, K., K.H. Nealson, and K. Horikoshi. 2004c. *Hydrogenimonas thermophila* gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing chemolithoautotroph within the *ε-Proteobacteria*, isolated from a black smoker in a Central Indian Ridge hydrothermal field. *International Journal of Systematic and Evolutionary Microbiology* 54:25–32, http://dx.doi.org/10.1099/ijs.0.02787-0.

Takai, K., M. Suzuki, S. Nakagawa, M. Miyazaki, Y. Suzuki, F. Inagaki, and K. Horikoshi. 2006b. *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* 56:1,725–1,733, http://dx.doi.org/10.1099/ijs.0.64255-0.

Taylor, C.D., C.O. Wirsen, and F. Gaill. 1999. Rapid microbial production of filamentous sulfur mats at hydrothermal vents. *Applied and Environmental Microbiology* 65:2,253–2,255.

Tuttle, J.H., C.O. Wirsen, and H.W. Jannasch. 1983. Microbial activities in the emitted hydrothermal waters of the Galapagos Rift vents. *Marine Biology* 73:293–299.

Vetriani, C., Y.S. Chew, S.M. Miller, J. Yagi, J. Coombs, R.A. Lutz, and T. Barkay. 2005. Mercury adaptation among bacteria from a deep-sea hydrothermal vent. *Applied and Environmental Microbiology* 71:220–226, http://dx.doi.org/10.1128/AEM.71.1.220-226.2005.

Vetriani, C., M.D. Speck, S.V. Ellor, R.A. Lutz, and V. Starovoytov. 2004. *Thermovibrio ammonificans* sp. nov., a thermophilic, chemolithotrophic, nitrate ammonifying bacterium from deep-sea hydrothermal vents. *International Journal of Systematic and Evolutionary Microbiology* 54:175–181, http://dx.doi.org/10.1099/ijs.0.02781-0.

Von Damm, K.L. 1990. Seafloor hydrothermal activity: Black smoker chemistry and chimneys. *Annual Review of Earth and Planetary Sciences* 18:173–204, http://dx.doi.org/10.1146/annurev.ea.18.050190.001133.

Von Damm, K.L. 1995. Controls on the chemistry and temporal variability of seafloor hydrothermal fluids. Pp. 222–247 in *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geochemical Interactions*. S.E. Humphris, R.A. Zierenberg, L.S. Mullineaux, and R.E. Thomson, eds, Geophysical Monograph Series, vol. 91, American Geophysical Union, Washington, DC.

Voordeckers, J.W., V. Starovoytov, and C. Vetriani. 2005. *Caminibacter mediatlanticus* sp. nov., a thermophilic, chemolithoautotrophic, nitrate-ammonifying bacterium isolated from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. *International Journal of Systematic and Evolutionary Microbiology* 55:773–779, http://dx.doi.org/10.1099/ijs.0.63430-0.

Wang, F., H. Zhou, J. Meng, X. Peng, L. Jiang, P. Sun, C. Zhang, J.D. Van Nostrand, Y. Deng, Z. He, and others. 2009. GeoChip-based analysis of metabolic diversity of microbial communities at the Juan de Fuca Ridge hydrothermal vent. *Proceedings of the National Academy of Sciences of the United States of America* 106:4,840–4,845, http://dx.doi.org/10.1073/pnas.0810418106.

Wankel, S.D., L.N. Germanovich, M.D. Lilley, G. Genc, C.J. DiPerna, A.S. Bradley, E.J. Olson, and P.R. Girguis. 2011. Influence of subsurface biosphere on geochemical fluxes from diffuse hydrothermal fluids. *Nature Geoscience* 4:461–468, http://dx.doi.org/10.1038/ngeo1183.

Wirsen, C.O., J.H. Tuttle, and H.W. Jannasch. 1986. Activities of sulfur-oxidizing bacteria at the 21°N East Pacific Rise vent site. *Marine Biology* 92:449–456.

Woyke, T., G. Xie, A. Copeland, J.M. Gonzalez, C. Han, H. Kiss, J.H. Saw, P. Senin, C. Yang, S. Chatterji, and others. 2009. Assembling the marine Metagenome, one cell at a time. *PLoS One* 4(4):e5299, http://dx.doi.org/10.1371/journal.pone.0005299.

Xie, W., F. Wang, L. Guo, Z. Chen, S.M. Sievert, J. Meng, G. Huang, Y. Li, Q. Yan, S. Wu, and others. 2011. Comparative metagenomics of microbial communities inhabiting deep-sea hydrothermal vent chimneys with contrasting chemistries. *The ISME Journal* 5:414–426, http://dx.doi.org/10.1038/ismej.2010.144.

Yamamoto, M., S. Nakagawa, S. Shimamura, K. Takai, and K. Horikosji. 2010. Molecular characterization of inorganic sulfur-compound metabolism in the deep-sea epsilonproteobacterium *Sulfurovum* sp. NBC37-1. *Environmental Microbiology* 12:1,144–1,153 http://dx.doi.org/10.1111/j.1462-2920.2010.02155.x.

Zhang, Y., I. Thiele, D. Weekes, Z. Li, L. Jaroszewski, K. Ginalski, A.M. Deacon, J. Wooley, S.A. Lesley, I.A. Wilson, and others. 2009. Three-dimensional structural view of the central metabolic network of *Thermotoga maritima*. *Science* 325:5,947, http://dx.doi.org/10.1126/science.1174671.