Deep-Sea Hydrothermal Vents: Potential Hot Spots for Natural Products Discovery?[⊥]

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Deep-sea hydrothermal vents are among the most extreme and dynamic environments on Earth. However, islands of highly dense and biologically diverse communities exist in the immediate vicinity of hydrothermal vent flows, in stark contrast to the surrounding bare seafloor. These communities comprise organisms with distinct metabolisms based on chemosynthesis and growth rates comparable to those from shallow water tropical environments, which have been rich sources of biologically active natural products. The geological setting and geochemical nature of deep-sea vents that impact the biogeography of vent organisms, chemosynthesis, and the known biological and metabolic diversity of Eukarya, Bacteria, and Archaea, including the handful of natural products isolated to date from deep-sea vent organisms, are considered here in an assessment of deep-sea hydrothermal vents as potential hot spots for natural products investigations. Of critical importance too are the logistics of collecting deep vent organisms, opportunities for re-collection considering the stability and longevity of vent sites, and the ability to culture natural product-producing deep vent organisms in the laboratory. New cost-effective technologies in deep-sea research and more advanced molecular techniques aimed at screening a more inclusive genetic assembly are poised to accelerate natural product discoveries from these microbial diversity hot spots.

Introduction

It is well recognized that natural product-based drugs provide a foundation of chemotherapy and trace back to the use of terrestrial plants and intertidal marine algae as traditional medicines thousands of years ago.[1] As technologies have advanced, the search for new natural product sources of biologically active compounds has expanded from terrestrial plants to microbes, to shallow water reef marine algae and invertebrates with their associated symbionts, ocean sediment-derived marine microbes, and even mine waste extremophiles.[2,3] Screening of phylogenetically diverse and unique organisms from rare or extreme ecosystems is a rational approach to discover novel chemotypes with medicinally relevant biological activities.[4] An unforeseen biological (especially microbial) diversity representing a largely untapped reservoir of genetic and metabolic heterogeneity continues to yield a wealth of new chemistry from these sources in ample demonstration of the value of natural products in drug discovery efforts.[5−7] Furthermore, natural products such as colchicine and kainic acid have played a critical role as research tools for use as molecular probes to dissect biological mechanisms and reveal new biochemical targets.[8] In the recent literature, the deep sea has emerged as a new frontier in natural products chemistry[9] at a time when there is a dire need for new drug templates to combat the escalating problem of drug resistance, especially in infectious diseases and cancer.

The deep ocean may be defined technically as depths beyond the euphotic zone (upper 200−300 m),[10] where the sea bottom, in darkness, receives less than 1% of organic matter from photosynthetic primary production, oxygen levels and temperatures (down to 2 °C) plummet, and hydrostatic pressures rise to greater than 1000 atm in the deep trenches (10 m water = 1 atm). However, in the realm of natural products chemistry, many logically report depths beyond those readily accessible by scuba as "deep". Thus, in a recent, comprehensive deep-sea review, Skropeta considered the range of ocean environments below 50 m (∼164 ft),[9] which host a variety of marine invertebrates and microbes adapted to physical extremes in environmental conditions. The introduction to the latter review provides an informative overview of the deep-sea environment and the effects of the extreme, although very stable, conditions on the gene regulation, macromolecules, and the metabolism of deep-sea organisms.

Once thought to comprise a very low diversity of organisms evolved to occupy a physiologically challenging niche that precluded the intense competition of many shallow-water ecosystems, deep-sea benthic communities are now recognized to be highly diverse, although not abundant. In the 1960s, focused efforts to sample the deep ocean floor resulted in unexpected findings of high faunal diversity, even in individual benthic dredge and epi-benthic sled samples from less than 100 to greater than 5000 m.[11] Nevertheless this high diversity, which is on the same order as that found in shallow tropical seas and has been attributed to the seasonal and geological stability of the deep-sea environment, occurs in relatively sparse pockets of slow-growing benthic organisms that are likely limited in density by food scarcity. Therefore, the exceptionally dense and diverse communities within the immediate vicinity of hydrothermal vents were in stark contrast to the surrounding sea bottom when first observed: in 1977, scientists aboard the manned deep submergence vehicle (DSV) *Alvin* dove in the Galapagos Rift valley (ca. 2500 m) to investigate recently photographed communities of large suspension-feeding benthic organisms surrounding active hydrothermal vents.[12] Hydrothermal vents, considered one of the most extreme environments on Earth, are formed when water heated in Earth's crust by magma is forced explosively to the surface through rock fissures in volcanic regions. At deep-sea vent sites, in addition to physical extremes of temperature (up to 400 °C) and pressure and a complete absence of light, there are also extremely steep chemical, pH, and temperature gradients between vent fluids and the surrounding seawater.[13] Remarkably, the growth rates of the deep vent communities proved comparable to organisms from shallow tropical environments,[14] and ultimately, the presence of extremely high concentrations of chemosynthetic microorganisms led to a new paradigm for primary production in the absence of sunlight.[15,16] Noteworthy is that the discovery of chemosynthetic symbiosis at deep-sea vent sites led to the realization that this phenomenon occurs in a wide range of habitats worldwide, typically characterized by high sulfide concentrations and the presence of free-living macroorganisms with reduced digestive systems. This diversity of chemosynthetic habitats, as well as their hosts and symbionts, is engagingly reviewed by

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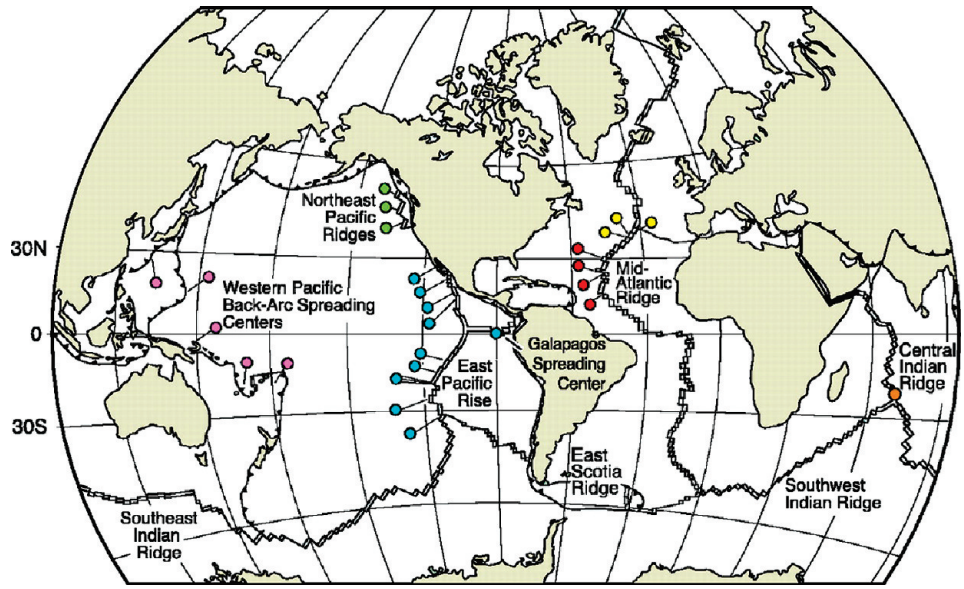


Figure 1. Map showing the major mid-ocean ridges and known deep-sea hydrothermal vent biogeographic provinces: Pink, western Pacific; green, northeast Pacific; blue, East Pacific Rise; yellow, Azores; red, Mid-Atlantic Ridge; orange, Indian Ocean. Reproduced with permission from Van Dover et al.[10]

Dubilier et al.[17] and includes sewage outfalls, organic-rich mud flats, some shallow-water coastal sediments, whale and wood falls in the deeper ocean, cold seeps, mud volcanoes, and continental margins, in addition to hydrothermal vents.

Deep-sea hydrothermal vents, besides being extreme environments, also represent some of the most dynamic environments on Earth. With unpredictable temperatures, chemical concentrations, flow dynamics, and seasonality, a single vent field may often be comprised of completely different fauna from one visit to the next.[14] As the vent matures and ages, early colonizers are superseded by a succession of other species in parallel with the diminishing thermal flow volume, temperature, and amount of hydrogen sulfide.[18] A distinction has been made between deep-sea and shallow-water hydrothermal vents on the basis of their biota. Tarasov et al. have shown a striking change in hydrothermal vent communities at depths of 200 m (660 ft) based on the occurrence of obligate vent fauna.[19] Thus, for the purpose of this review, deep-sea hydrothermal vents are those that occur below 200 m.

In order to discuss deep-sea hydrothermal vents as potential hot spots for natural products investigations, here we consider the geological setting and geochemical nature of deep-sea vents that impacts the biogeography of vent organisms, chemosynthesis, and the known biological and metabolic diversity of eukaryotes and prokaryotes at vent sites and the handful of small molecule natural products isolated to date directly from deep-sea vent organisms. Of critical importance too are the logistics of collecting deep vent organisms, opportunities for re-collection, considering the dynamic nature of vent sites, and the ability to culture natural product-producing deep vent organisms in the laboratory.

Geology and Distribution of Deep-Sea Hydrothermal Vents

Currently, there are over 300 deep-sea hydrothermal vent sites known throughout the world.[20] These vent sites generally occur along a nearly continuous underwater mountain chain (midocean ridges) totaling more than 75 000 km that remains largely unexplored for hydrothermal activity (Figure 1).[18] Located at the boundaries between the tectonic plates of Earth's crust, these midocean ridges are the sites of incremental seafloor spreading (spreading centers) at which molten rock (magma) rises toward Earth's surface as the tectonic plates move in relation to each other. Hydrothermal vent fields may comprise multiple zones of focused hot and diffuse (low-temperature) fluid flows and range in size from several hundred to several million square meters around ridge axes. Hydrothermal vents are also found behind island arcs along active plate margins in "back-arc spreading centers" and active submarine volcanoes or seamounts located in the center of tectonic plates.[21] As a result of their proximity to the countries primarily involved in deep-sea hydrothermal vent research, the most studied hydrothermal systems are either in the eastern Pacific (East Pacific Rise and the Juan de Fuca, Gorda, and Explorer Ridges) or the north-central Atlantic (northern Mid-Atlantic Ridge). The distinct geological settings of different hydrothermal vents impact the extent of venting on both spatial and temporal scales and thus influence the biogeography of vent organisms. Therefore, variations in midocean ridge crest dynamics between different ocean basins, as well as regional and local differences in ridge morphology (valley depth, etc.), which affect bottom currents, style of venting, and vent longevity, make it important to keep in mind the geographical context of the general descriptions of vents and their biota presented here.

Vents are typically characterized by the mineral composition of their emissions and the morphology of structures built up through mineral deposition: variations in fluid composition, temperature, mineralogy, and shape and size of deposits exist.[22] As cold seawater penetrates deep into Earth's crust, it is heated and chemically modified from interactions with very hot basaltic rock.[23] Hot fluids, exiting vents at up to 400 °C, are enriched with transition metals (e.g., aluminum, copper, cobalt, iron, lead, manganese, and zinc), silica, sulfides, and dissolved gases such as hydrogen and methane.[18,21] The rapid mixing of these hydrothermal fluids with the surrounding cold seawater as they exit from the ocean floor causes changes in pH and temperature and the precipitation of metal sulfides and minerals to form particle-rich black plumes and columnar sulfide-rich *black-smoker chimney* structures. At many active vent sites, within a year of a volcanic eruption, mature (>5 m) black-smoker sulfide chimneys are observed that eventually grow 10−20 m high and may have several high-temperature orifices near the top.[22,24] *White-smoker chimneys* form around intermediate temperature flows (100−300 °C) that facilitate the precipitation of silica, anhydrite, and Barite as white particles. In addition, there are several other structural variations of sulfide-rich mineral deposits, including beehives (with horizontal layering and conduits for diffuse fluid flow), flanges (where pooled hot fluids are trapped

beneath the shelf-like structure), and complex sulfide mounds.[18] The complex mineralization processes ongoing in even mature sulfide structures result in convoluted internal plumbing that may create diffuse warm-water flows at temperatures and mineral fluxes suitable for the growth of organisms. In addition to issuing from porous surfaces of active mineral deposits, low-temperature, diffuse flows may also exit directly from fissures in basalt lavas. One of the largest known hydrothermal deposits, "Godzilla", is an extreme example of a complex sulfide mound.[25] This sulfide structure has a diameter of 12−20 m and towers 55 m (180 ft) above the valley floor of the High-Rise vent field, Juan de Fuca Ridge. Hydrothermal fluids from 30 to 330 °C exit at various tiers of its flanges extending 4 to 5 m (13−16 ft) away from the structure. The extent of this venting may be brought into perspective by the observation that it is common to measure temperature changes from 350 to 10 °C over a distance of just a few centimeters around the majority of high-temperature vent orifices at deep-sea hydrostatic pressures.

In addition to the particular morphology of a deep-sea vent, temperature and chemistry fluxes and the duration of hydrothermal activity have a profound effect on the composition of vent communities. Where rates of seafloor spreading are more rapid, as on the East Pacific Rise, eruptive disturbances are frequent enough that individual chimneys or diffuse-flow areas in a vent field may be present for less than 20 years. Alternately, in areas where volcanism is less frequent, such as the Main Endeavor Field (Juan de Fuca Ridge), some active mounds are thought to be more than 200 years old.[18]

Biogeography and Diversity of Deep Vent Eukaryotes

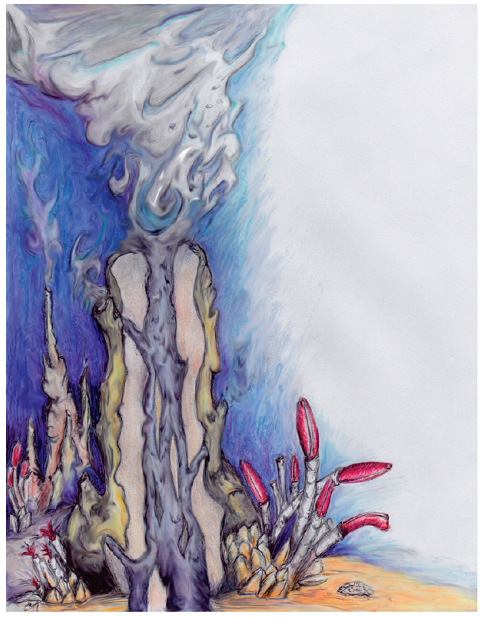
The dense invertebrate communities typically associated with deep-sea hydrothermal vents exist in diffuse, warm-water flows that sustain temperatures of 10−40 and occasionally up to 60 °C.[18] Despite the high biomass associated with hydrothermal vents, there is much lower macrofaunal species diversity relative to other deep-sea communities. This is likely a result of the dynamic and variable fluid conditions both within and between vent habitats that require specialized physiological and biochemical adaptations[14,18] and favor the emergence of dominant species that succeed in a range of fluid conditions.[26] However, the full extent of the species present within hydrothermal vent communities has yet to be discovered considering the vast unexplored ocean ridge systems and the report of new species being described every two weeks throughout the 1990s.[14]

Over 500 eukaryote species, encompassing 12 animal phyla and more than 150 new genera, have been described in the last three decades from deep vent sites. Arthropods (38.8%), mollusks (28.6%), and annelid worms (17.7%) dominate the megafaunal vent communities throughout the world, while cnidarians (4.6%), chordates (3.7%), and sponges (1.9%) are of notable presence.[20] Although deep ocean currents can disperse larval organisms over vast distances to new hydrothermal fields, many hydrothermal vent fields exhibit a unique range of habitat diversity and a high degree of endemism.[18,21] The Galapagos Rift and East Pacific Rise of the Pacific Ocean have similar communities, whereas different vent communities on the Juan de Fuca Ridge (northeast Pacific) share few species.[27] During a visit to a mature hydrothermal vent site in the eastern Pacific, one might expect to observe scattered aggregations or "bushes" of siboglinid polychaetes (e.g., the vestimentiferan tube worms *Riftia pachyptila* or *Ridgeia pisceae*), which, on closer inspection, host a mix of limpets and snails, alvinellid polychaetes (e.g., the palm worm *Paralvinella palmiformis*), and polynoid polychaetes (e.g., the scale worm *Lepidonotopodium piscesae*), all cloaked in a white microbial mat, with occasional hydrothermal vent shrimp and Yeti crabs. On the Mid-Atlantic Ridge, vent sites are characterized instead by an abundance of hydrothermal vent shrimps (*Rimicaris*) swarming over chimneys near high flows that lack the vestimentiferans and alvinellids of the Pacific vent communities.[18] Nonetheless, at least 40% of the Atlantic genera reported are shared with the Pacific vent fauna. In both oceans, beds of hydrothermal vent clams (*Calyptogena magnifica*) or mussels (*Bathymodiolus thermophilus*) may be observed in lower flow areas. The Kairei and Edmond vent fields of the Indian Ocean contain genera shared with either Atlantic or Pacific vents. However, Indian Ocean communities are different enough to constitute a separate biogeographic province from either the Atlantic or Pacific.[28]

Unlike communities in other deep-sea reducing habitats (e.g., cold seeps), hydrothermal vent communities change on time-scales of months to years, and frequent volcanic activity along ridge systems with medium to fast seafloor spreading rates may continually reset vent community development, allowing repeated primary successions to be documented in conjunction with geophysical and chemical parameters.[29] Within two years of the eruption of Cleft Segment on the Juan de Fuca Ridge (eastern Pacific), tubeworm-associated assemblages were well-established at diffuse vents. These communities were observed almost annually over the next six years (2−8 years post-eruption) and showed a shift in dominance from the alvinellid polychaete *Paralvinella pandorae* (20−98% of the assemblages at year 2) to the limpet *Lepetodrilus fucensis* by year 7. Most diffuse venting had waned by year 5, and all vents were extinct by year 8. In another more recent report on succession in diffuse-flow vent communities after the 1998 eruption at Axial Volcano (central segment, Juan De Fuca Ridge), Marcus et al. first examined mature pre-eruption communities using data for 21 low-temperature vents that were sampled in Axial vent fields in 1986−1988 and 1997−1999.[26] Species composition proved very similar among mature vents, and the collections were dominated by a small subset of taxa, although the relative dominance of the common species depended on vent flow temperature. As observed in earlier studies, alvinellid worms dominate higher temperature vents, while limpets dominate lower temperature vents (<18 °C). This predictability of mature vent communities bodes well for natural products investigations that require re-collections to pursue preliminary results from broad biological activity profiling of initial small-scale, diverse collections.

Post-eruption colonization of new vents depends on the species pool available to colonize a site, as well as the physicochemical characteristics of a particular vent environment. Marcus et al. found that *Paralvinella pandorae* colonized and dominated the new Axial vent communities within the first year post-eruption.[26] *Ridgeia piscesae* tubeworms took up to 30 months to become well-established, by which time 23 of the 36 known Axial vent and four new macrofaunal species had arrived. This may coincide with the tapering of high vent flows to a level where temperature and hydrogen sulfide levels were acceptable to juvenile recruits that require both oxygen and sulfide. Interestingly, some rare macrofaunal species showed rapid recruitment and colonization, although they never reached significant abundance. By year 2 after the eruption, biomass of tubeworm-associated species had leveled, but animal densities remained significantly higher than at the mature vents, suggesting that the latter support larger individuals. This knowledge of the rates of colonization, progressive succession, and ecology of vent communities at different sites may be critical to the success of natural products investigations that must rely on limited collection opportunities, both spatially and temporally.

Eukaryotic microorganisms from both known and previously undescribed taxa have also been described from deep-sea hydrothermal and cold-seep environments, including those living in association with various invertebrate species. Sequence comparisons of PCR-amplified small-subunit (SSU) rRNAs were used to characterize the diversity of eukaryotic organisms associated with hydrothermal sediments from the Guaymas Basin in the Gulf of California.[30] Many of these sequences are related to previously uncharacterized eukaryotes or seem to represent early branching within well-characterized eukaryotic clades. These include sequences from certain fungi, green algae, diatoms, water molds,



Sample Description	Temp. (°C)	Total Bacteria Cell Count	Phylogenetic Clusters ([a]OTUs)
hydrothermal plume[126]	~200	6.3×10^4 – 7.5×10^4 cells mL^{-1}	4 (20)
hydrothermal fluid[72]	2–208	[b]ND	15 (46)
[c]ISCD (5 days)[59,127]	2–300	6.8×10^4 – 2.0×10^7 cells g^{-1}	7 (22)
chimney structure enrichment cultures[110]	ND	2.2×10^9 cells mL^{-1}	9 (17)
tubeworm (*Riftia pachyptila*)[128]	ND	ND	8 (39)
polychaete worms (*Paralvinella palmiformis*)[74]	ND	ND	8 (93)
scaly snail (*Crysomallon squamiferum*)[44]	ND	ND	5 (76)
microbial mat[129]	~37	ND	3 (18)
hydrothermal sediment[53]	ND	ND	12 (59)
hydrothermal sediment cores[73]	2–74	ND	9 (36)

Figure 2. Representative bacterial counts and phylogenetic diversity reported from different hydrothermal vent niches as labeled. Note that the data referenced are from different geographical locations. [a]Number of operational taxonomic units (OTUs) determined within referenced material; [b]ISCD, *in situ* colonization device; [c]ND, not determined.

protists, acanthareans, and radiolarians. Obviously, phytoplanktonic taxa, such as green algae and diatoms, could have been deposited through sedimentation from the euphotic zone, and culture-independent molecular surveys do not necessarily reflect viable members within sediment samples. However, Atkins et al. isolated and cultured 18 strains of flagellated protists representing nine species from four deep-sea hydrothermal vent sites, including the Guaymas Basin.[31] This suggests that many of these sequences represent truly unique eukaryotic microorganisms capable of adapting to life in extreme and dynamic environments. In addition, the Deep-sea Microorganism Research Group at the Japan Marine Science and Technology Center (JAMSTEC) has identified new species of unicellular fungi, typically designated as yeasts, associated with the tubeworm *Lamellibranchia* sp. and the giant white clam *Calyptogena* sp.[32,33] These organisms live in cold-seep environments where high concentrations of methane and hydrogen sulfide flow from the seafloor.

The dominant and ubiquitous vent species highlighted here (arthropods, gastropods, and annelids) belong to taxa widely recognized from other environments not to be prolific producers of natural products. However, the vast majority of vent invertebrates host epibiotic or endobiotic extracellular or intracellular symbionts and, in this respect, could be viewed as the equivalent of shallow-water filter feeders such as sponges and tunicates, which are both responsible for tremendous natural products diversity from symbiotic microbes that account for up to 40% of their body mass.[34] Thus, the crucial question becomes the biological and metabolic diversity of the symbiotic, and also free-living, microorganisms associated with the dense vent invertebrate communities (Figure 2), which in fact support primary production in those vent communities via chemosynthesis. In addition to providing critical food resources to their hosts, epi- and endosymbiotic bacteria have been proposed as sources of natural products for deterring predation within these communities.[35]

A variety of evidence indicates that nearly all invertebrates associated with hydrothermal vents acquire much if not all of their needs for fixed carbon and nitrogen from microbial symbionts, which are dominated by sulfide-oxidizing bacteria that require a constant supply of sulfide to specialized host tissues.[36] For example, adult vestimentiferan tubeworms lack a digestive tract and derive their nutrition solely from culturing sulfur-oxidizing bacteria within a specialized organ known as the trophosome.[37,38] The trophosome accounts for approximately 16% of the animals' wet weight and consists primarily of symbiont-containing lobes (bacteriocytes), crystals of elemental sulfur, and blood vessels.[14,37] Bacterial densities between 10^9 and 10^{11} cells per gram of wet tissue have been observed within the trophosome of the giant vestimentiferan tubeworm, *Riftia pachyptila*, which is capable of growing up to 2.0 m in length.[20] Although numerous reports indicate dominance by only a single bacterial phylotype within trophosomes of *R. pachyptila* and other vestimentiferans, new molecular evidence suggests that a more diverse community may colonize these and other structures within the trunk of these tubeworms.[39,40] In general, the potential diversity of chemosynthetic symbionts, which may arise from many different bacterial lineages, has only recently been appreciated with the advent of molecular methods that have revealed a remarkable variety of chemosynthetic metabolic pathways[17] discussed in more detail in the following section. Other examples of vent symbioses include suspension-feeding hydrothermal vent clams and mussels, which gain approximately 45% of their fixed carbon from chemoautolithotrophic (auto = fixing inorganic carbon; litho = oxidation of inorganic electron donors) microorganisms associated with their gills.[14,41] A highly diverse assemblage of episymbionts was identified from the dorsal surface of the extremely thermotolerant polychaete *Alvinella pompejana*.[42] Other epibiont communities include microbes "farmed" on dense aggregations of shrimp at Mid-Atlantic Ridge hydrothermal vents. The shrimps compete for space near warm, sulfide-rich water emissions to support their "crop" of microorganisms.[43] Microorganisms are also associated with iron sulfide-containing sclerites in the foot of a newly described scaly snail, *Crysomallon squamiferum*, from hydrothermal vents in the Indian Ocean.[44] Without these chemosynthetic microorganisms, the rapid growth rates required to prosper and reach reproductive maturity in such an extreme and dynamic environment would not be possible.[14,45]

Chemosynthesis and the Biogeography and Diversity of Archaea and Bacteria

At deep-sea hydrothermal vents, in the absence of light and the presence of hydrothermal fluids rich in minerals, reduced com-

pounds (including H_2S, CH_4), and CO_2, chemical energy replaces solar energy as the fuel that supports primary production by chemosynthetic bacteria and archaea.[18,36] The Archaea comprise a distinct domain of microorganisms that have no cell nucleus or membrane-bound organelles (the same as "prokaryotic" Bacteria), but possess unique biochemistry and have several metabolic pathways that are more closely related to those of eukaryotes (especially transcription and translation).[46] Bacteria and archaea may be suspended in the ambient water column or hydrothermal plumes or attached to rocks, to sediment, or on/in vent animals, which in turn may feed directly on the microbes or engage in symbiotic associations to acquire fixed carbon and nitrogen, as discussed in the previous section.[22,24,25] Figure 2 provides a representation of the different vent habitats for microorganisms, with representative bacterial counts and taxonomic diversity reported for each niche.

Gradients of temperature and chemistry (including O_2) in hydrothermal systems support free-living microorganisms with a diverse array of obligate and facultative physiologies and tolerances, and thus microbial diversity correlates closely with major element and volatile chemistry of vent fluids. It has been proposed that individual microorganisms switch freely between autotrophy (inorganic carbon energy sources) and heterotrophy (organic carbon energy sources) depending on environmental conditions, since heterotrophy yields higher energy when available. However, in the absence of a supply of small organic compounds supporting heterotrophic metabolism, chemosynthetic microorganisms produce particulate organic carbon for vent communities and are important as sinks for reduced hydrothermal compounds in the global cycling of elements.[18] Since the concentration of H_2S in vent fluids is extremely high (3 to 110 mmol per kg seawater), sulfide oxidation is a dominant microbial chemosynthetic source of energy in vent communities.[36] The oxidation of reduced compounds such as HS^-, H_2S, S^0, CH_4, H_2, and NH_4^+ or Fe(II)- and Mn(II)-containing minerals provides energy for the synthesis of useable organic carbon from inorganic sources such as CO_2 and CH_4.[18,47] The fixation of CO_2 used by many of these chemoautolithotrophic bacteria is identical to the Calvin−Benson cycle used by plants. While aerobic microbes use O_2 as the electron acceptor during the energy-yielding chemosynthetic reaction, anaerobic hydrothermal microorganisms use CO_2, Fe^{3+}, NO_3^{2-}, or organic compounds (to oxidize H_2).[36,48]

Free-living bacteria and archaea are suspended within buoyant vent plumes at temperatures reaching at least 115 °C, and cell densities several orders of magnitude more abundant than surrounding seawater can be detected hundreds of kilometers away from vent fields.[16,49] These microorganisms also form microbial mats of various colors and morphologies on the surface of basalt[50,51] and chimney spires[52] and within hydrothermal sediments,[53] where they serve as food for numerous invertebrate filter-feeding, grazing, and deposit-feeding species.[54] They likely also play a significant role in early steps of macrofaunal colonization around new vent formations.[55,56] Many anaerobic microbes are also found deep within the thermal subseafloor[57] and chimney walls in close proximity to nutrient-rich, acidic effluent that can vary in temperature from ≤25 to 350 °C.[58] A steep temperature gradient exists within only a few centimeters of these walls, where variations in the abundance and diversity of bacteria and archaea occur. Numerous studies have shown that hyperthermophilic archaea increasingly dominate the microbial consortium as effluent temperatures rise from 150 to 300 °C,[59−62] although cell densities of both archaea and bacteria decrease in proximity to such extreme temperatures. It should be noted that the current upper limit for hyperthermophiles isolated in culture is 115 °C, although Baross and Deming have reported (unreplicated) evidence for a consortium of growing "superthermophiles" in fluids collected from black smokers (the hottest vents) and maintained in culture at 150−250 °C under 265 atm.[63]

Although there has been tremendous scientific interest in the microbial ecology of "hot-spot ecosystems", such as hydrothermal vents, cold seeps, and gas-hydrate systems, the distribution and diversity of functional and taxonomic groups of bacteria and archaea within the deep sea is largely unknown.[64] The diversity of hydrothermal vent microbial communities cannot truly be assessed by methods that rely solely on artificial cultivation, since 99% of marine microbes are considered unculturable.[65] These challenges have been overcome in part by the application of a molecular phylogeny-based approach using nucleotide-sequence analysis of the highly conserved gene for the small-subunit (SSU) rRNA molecule (16S rRNA).[66] This approach has revealed that the global diversity of microorganisms is at least 100 times greater than estimates based on cultivation-dependent surveys; new phylotypes, often representing major new lineages, are consistently shown with each molecular analysis of microbial environments.[66−68] For example, the new archaeal phylum "Nanoarchaeota"[69] has been identified by analysis of PCR-amplified SSU rRNA genes from a defined coculture of hyperthermophilic archaeans, and similar methods have indicated the emergence of a newly defined lineage distributed throughout the global deep-sea vent system referred to as the "Deep-Sea Hydrothermal Vent Euryarchaeoic group" (DHVEG).[61]

At the Josephine Bay Paul Center, Marine Biological Laboratory (Woods Hole, MA), and the Joint Institute for the Study of Atmosphere and Ocean, University of Washington (Seattle, WA), an alternative approach to the more traditional analysis of full-length 16S rRNA amplicons has been developed.[67] This approach targets hypervariable regions within 16S rRNAs that can record differences between both divergent and closely related organisms and thus provide a greater resolution of microbial diversity and relative abundance.[67,70] Analysis of 689 720 bacterial and 216 627 archaeal amplicons, targeting the V6 hypervariable region, from two low-temperature (∼30 °C) diffuse-flow vents in the northeast Pacific Ocean revealed 30 108 unique bacterial sequences forming 18 537 phylotypes and 5979 unique archaeal tag sequences defining 1931 phylotypes.[71] By comparison, previous assessments from the same sites using traditional PCR-amplified 16S rRNA gene sequence analysis identified only 55 bacterial and 28 archaeal phylotypes [operational taxonomic units (OTUs)].[69,72] Even more remarkably, this attempt by Huber et al.[71] to provide an exhaustive characterization of bacterial and archaeal diversity at the study sites was not successful in the case of the bacteria: statistical analyses of the data from even this unparalleled number of bacterial sequences indicated additional, undescribed bacterial diversity at every taxonomic level. Hence, much of the microbial diversity reported from these environments is likely at best a conservative approximation of their true community structure. This strategy of sequencing the 16S rRNA V6 hypervariable region has also been adopted by the International Census of Marine Microbes, one of 14 groups within the Census of Marine Life (CoML) initiative. The goal of the CoML is to provide an online database (first release in 2010) that describes each one of the more than 14 million marine species currently known. Since its inception in 2000, researchers have discovered more than 5600 new species from near-shore habitats to the abyssal plains, including mid-ocean ridges and deep-sea vents.[64]

Bacterial diversity at deep-sea hydrothermal vents, as for nonthermal deep-sea environments, spans most of the currently defined lineages including the Actinobacteria (high G+C Gram positives),[73] Firmicutes (low G+C Gram positives encompassing the Bacilli, Clostridia, and Mollicutes),[53,72] and the Bacteroidetes (formerly *Cytophaga-Flexibacter-Bacteroides*) phylum.[57,74] The presence of Actinobacteria, some of which have larger genome sizes (over 9 Mb for some *Streptomyces* and *Rhodococcus* species), is particularly encouraging given that they are the most prolific source of natural product-derived medicines.[75] A correlation between

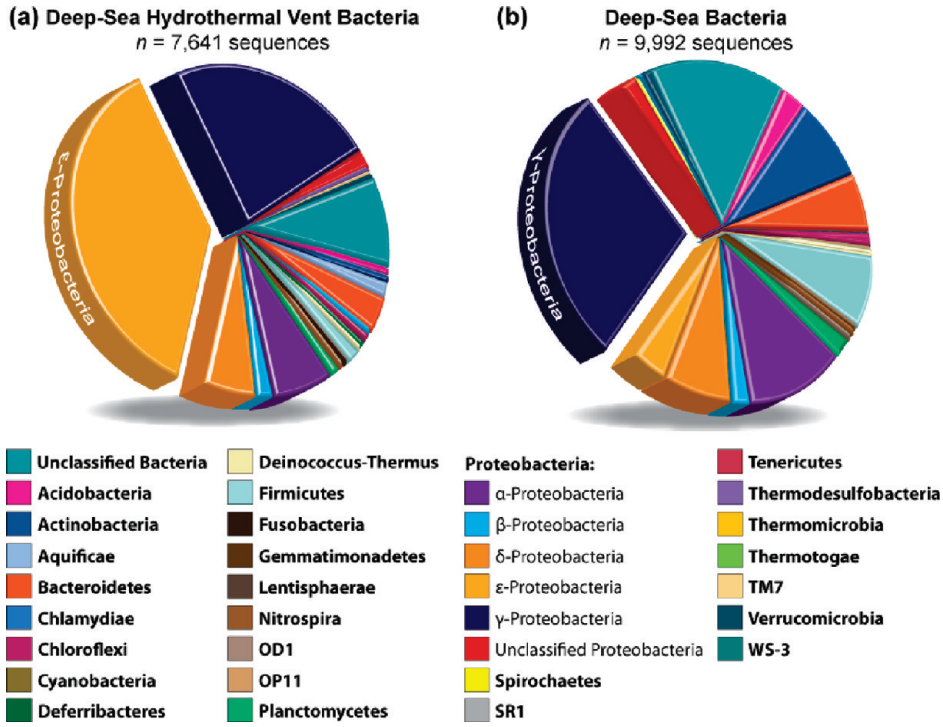


Figure 3. Relative abundances of bacterial phyla (including classes of Proteobacteria) found in the deep sea as determined by 16S rRNA gene sequence analysis and reported in the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/): (a) hydrothermal vent samples and (b) nonthermal water column and sediment samples.[130,131] Note that these data do not include 16S rRNA V6 hypervariable region sequences, but those data show comparable diversity and relative abundances of phylogenetic groups.[71]

genome size and secondary metabolic capacity has emerged in recent studies aimed at evaluating how relative usage of the genome varies with genome size. The functional characterization of 115 completed bacterial genomes in the Genbank database showed that the relative proportion of genes for regulation and secondary metabolism increases with genome size.[76]

Notably, the relative distribution of bacteria among taxonomic groups differs between deep-sea hydrothermal vent and nonvent environments (Figure 3). In fact, Actinobacteria form a relatively small portion of the known bacterial diversity at vents (Figure 3a) compared to other nonthermal environments (Figure 3b). However, the much higher concentrations of bacteria in hydrothermal versus cold sediments, for example,[73] should lead to greater sampling efficiency at hydrothermal vents and therefore favor these environments as a source of Actinobacteria as well as other likely natural product producers. The Proteobacteria (Gram negatives) form the largest bacterial phylum, comprising approximately one-third of all known bacteria, and represent a diverse range of organisms with varying genome sizes and life histories. This phylum includes sulfur-oxidizing bacteria (e.g., *Beggiatoa* species), methanotrophs (e.g., *Methylobacter* species), and nitrifying bacteria (e.g., *Nitrococcus* species) as well as bacteria responsible for animal bioluminescence (e.g., some *Vibrio* species). In addition, β-, γ-, and δ-subclasses of Proteobacteria include some gliding forms, predatory species of which have unusually large genome sizes and are thought to secrete antibiotics to weaken or immobilize their prey.[77]

The nonvent deep-sea bacteria comprise highest numbers of γ-proteobacteria followed by α-proteobacteria, Firmicutes, Actinobacteria, and δ-proteobacteria (Figure 3b). In contrast, ε-proteobacteria dominate deep-sea vent microbial communities, although other subclasses of Proteobacteria (γ-, α-, and δ-) and the Aquificae (a small group of thermophilic/hyperthermophilic chemolithotrophic bacteria) are also found widely in a variety of vent habitats including sulfide structures, hydrothermal fluids, sediments, and microbial mats.[78] The ε-proteobacteria subclass is ubiquitous in Nature and incorporates sulfur-metabolizing and microaerophilic bacteria, including many enteric mammalian pathogens (e.g., *Helicobacter* and *Campylobacter* species). The success of ε-proteobacteria, including a high degree of endemism to very specific habitats, is attributed in large part to the genomic plasticity of these bacteria, which lack many DNA-repair genes.[79] At hydrothermal vents, species of *Arcobacter*, *Sulfurovum*, *Sulforimonas*, *Hydrogenimonas*, *Nitratiruptor*, and many other genera play a key role in early steps of microbial and invertebrate colonization processes through the cycling of sulfur, hydrogen, nitrogen, and carbon at vent fields.[55,78,80] Although the known genome sizes of members of these genera fall between 1.8 and 2.6 Mb, some ε-proteobacteria have been observed as filamentous aggregations,[44,51,59,81] the formation of which may rely on chemical signaling. Notably, recent metagenomics analyses of water-column bacteria reveal that deep-sea bacterial communities have a larger average genome size than surface-dwelling species and are characterized by a higher metabolic diversity and genomic plasticity.[82]

The diversity of archaea within microbial communities associated with active chimney structures can vary from a single species (invertebrate symbionts and populations deep within chimney structures) to more complex communities. To date, phylotypes from every known and newly discovered marine archaeal group have been observed in deep-sea hydrothermal fields throughout the world (Figure 4a). While archaea are generally associated with small genome sizes (<2−3 Mb), methanogens of the genus *Methanosarcina* have genome sizes of 3 to 5.7 Mb and are found ubiquitously in diverse habitats. These unusual archaea possess all three known pathways for methanogenesis and can utilize nine different methanogenic substrates. They comprise a significant portion of the largely (formally) unclassified deep-sea archaea (Figure 4b) and have also been detected in hydrothermal sediments from the Guaymas Basin[73] and high-temperature chimneys in the Lost City Hydrothermal Field (Mid-Atlantic Ridge).[83]

Natural Products from Deep-Sea Vent Environments

The rate of discovery of new natural products from marine invertebrates and microorganisms appeared to have peaked in the late 1990s and to be declining at the turn of the century. However,

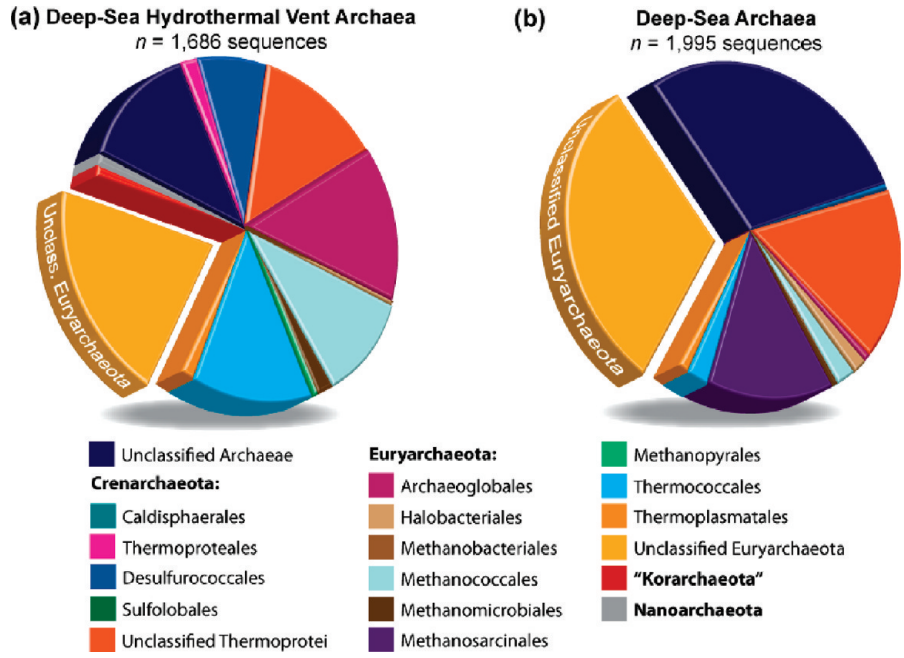


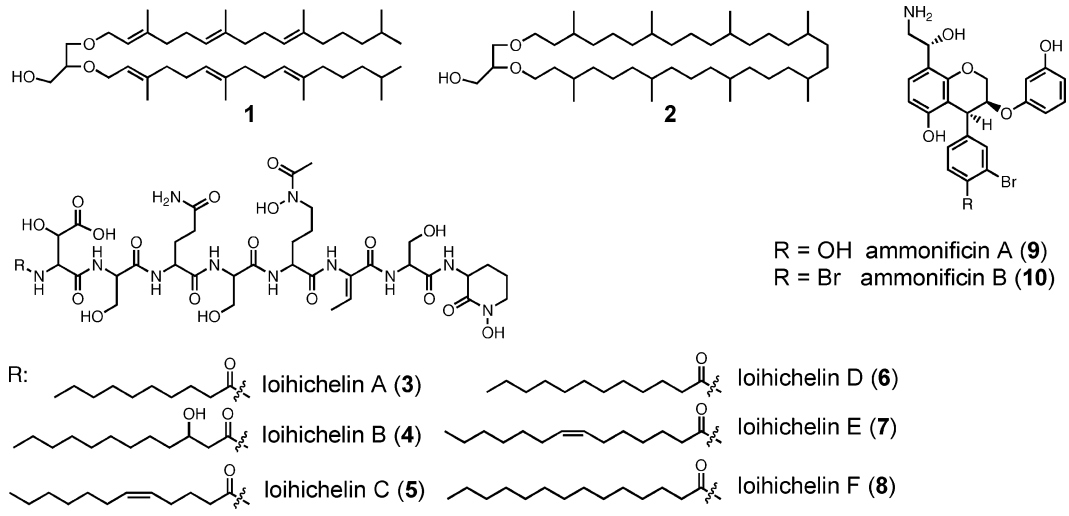
Figure 4. Relative abundances of phyla and component classes of archaea found in the deep sea as determined by 16S rRNA gene sequence analysis and reported in the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/): (a) hydrothermal vent samples and (b) nonthermal water column and sediment samples.[130,131] Note that these data do not include 16S rRNA V6 hypervariable region sequences, but those data show comparable diversity and relative abundances of phylogenetic groups.[71]

increasing numbers of new, biologically active marine natural products are once again being reported: 961 new compounds were reported in 2007 versus 779 new compounds in 2006.[84] This dramatic increase (24%) is attributable primarily to an increased focus on marine microorganisms. Furthermore, the continued dominance of sponges as a source of new compounds is consistent with the diverse assemblages of microbial symbionts associated with these readily collectable organisms, many compounds from which are of putative or proven microbial origin.[34,85] Microbial metabolites predominate among agents under development for the treatment of human diseases.[86] Actinomycete soil bacteria (order Actinomycetales) are the source of nearly 45% of all biologically active microbial metabolites and are responsible for over 50% of the microbial antibiotics discovered to date, most of which originate from the *Streptomyces* and *Micromonospora* genera.[75] When marine actinomycetes taxonomically related to known terrestrial genera were first isolated from shallow coastal sediments, they were largely believed to originate from dormant spores deposited in marine sediments from terrestrial runoff.[87,88] However, since the first description of an autochthonous marine actinomycete species, *Rhodococcus marinononascens*,[89] many obligate halophiles have been characterized, including many new taxa. Over the last 15 years, seminal research by Fenical and Jensen has firmly established actinobacteria from marine sediments as a valuable source of drug discovery leads.[87] Considering deep-sea vent environments, of specific interest is that new actinomycetes have been isolated from hydrothermal vent fluids in the Mariana Trough (ca. 2850 m) and Suiyo Seamount (ca. 1390 m)[90] and from hydrothermally active sediments of the Guaymas Basin (ca. 2005 m).[73] Furthermore, filamentous bacteria (possibly Actinobacteria) have been observed in the guts of vent invertebrates.[91]

In addition to the specialized equipment needed to survey and sample the ocean floor, the generally accepted pre-1960s paradigms that the deep ocean lacked invertebrate biological diversity and that most important groups of antibiotic-producing bacteria were not indigenous in the oceans delayed NPs investigations of the deep sea and also near-shore sediment-derived marine microbes. In the last 35 years, new, more cost-effective technologies in deep-sea research have resulted in literature reports of natural products from deep-sea cnidarians, sponges, echinoderms, bacteria, fungi, and archaea collected from bathyal (200−4000 m) and abyssal (4000−6500 m) ocean depths, as reviewed by Skropeta.[9] Nevertheless, the number of natural products from the deep sea (>200 m) is only 1−2% of the more than 20 000 marine natural products reported in the last fifty years. The Skropeta review provides a graphical profile of the numbers of new compounds isolated through 2007 by depth range (below 50 m), which shows 267 new marine compounds collected from depths below 200 m.[92] Of these, only two new molecules are attributable to deep-sea hydrothermal vent organisms, both archaea. In separate reports, anaerobic cultures of *Thermococcus* S 557 (cultured under N_2, 85 °C) and methanogenic *Methanococcus janaschii* (cultured under H_2, CO_2, 85 °C) produced isoprenyl glycerol ethers **1**[93] and **2**,[94] respectively. However, these and other known archaeal glycerol ethers may be classified as primary rather than secondary metabolites: archaea are characterized by the production of thermally and chemically stable ether-containing membrane lipids. The only deep vent *invertebrate* metabolites reported to date are a variety of known sterols from bivalves. These were obtained from *Bathymodiolus septemdierum* around hydrothermal vents at 1244 m in the Myojin Knoll at Izu-Bonin Island Arc and from *Calyptogena soyoae* clams collected from a deep cold seep (vent) at 1100 m in Sagami Bay, Japan.[95]

The 2009 reports of the loihichelins and the ammonificins may be considered to be the first publications on *new* natural products from deep-sea vent environments. Loihichelins A−F (**3**−**8**) are amphiphilic peptidic siderophores isolated from cultures of the heterotrophic bacterium *Halomonas* LOB-5 collected from Loihi Seamount (east of Hawaii, −1174 m)[96] and are related to the amphibactins, aquachelins, and marinobactins from ubiquitous marine bacteria.[97] With their octapeptide polar head groups and relatively short fatty acid moieties, the loihichelins are the most hydrophilic of the reported amphiphilic siderophores, which all chelate Fe(III) via the bidentate coordination of two hydroxamate groups and a β-hydroxyaspartate residue. Three 2 L cultures of the bacterium provided 8 mg of loihichelin C for 1D and 2D NMR spectroscopy. These data confirmed the structural information gained for all six loihichelins by amino acid analysis using Marfey's method, peptide sequencing by tandem mass spectrometry and fatty

Chart 1



acid analysis by GC-MS. *Halomonas* strains are broadly distributed in association with deep-sea volcanic weathered basalt and sulfide rocks in low-temperature hydrothermal vent fields and possess the functional capability of Fe(II) and Mn(II) oxidation.[96] It is not known whether the loihichelins serve a role only in the acquisition of iron as a trace nutrient or whether they are required for energy generation by *Halomonas* during the metabolism of reduced Fe(II).

Ammonificins A (**9**) and B (**10**) were reported from the chemolithotrophic bacterium *Thermovibrio ammonificans* (phylum Aquificae, order Aquificales), which was isolated from the walls of an active deep-sea hydrothermal vent chimney on the East Pacific Rise (9°50′ N).[98] Optimal growth of this thermophilic anaerobe is seen at 75 °C (pH 5.5, 2% w/v NaCl) in the presence of H_2 and CO_2 with nitrate or sulfur as the electron acceptor, which generates ammonium or hydrogen sulfide, respectively. A 5 L culture produced 40 g wet weight of bacterial mass from which 3 mg of **9** and 1.6 mg of **10** were isolated. These brominated hydroxyethylamine chroman compounds did not show biological activity in an apoptosis induction assay and gave inconclusive results in antimicrobial tests, possibly due to minor inseparable contaminants which could be responsible for the original activity of the parent extract.

It is widely accepted that the production of natural products represents a critical chemical defense mechanism adopted by organisms that lack physical/structural defenses against predation.[99] The occurrence of isolated pockets of dense vent communities in the vast expanse of sea floor otherwise devoid of standing biomass implies the presence of defense mechanisms against predation by generalist feeders. An obvious conclusion is that the extreme and dynamic nature of deep-sea vent habitats, including high levels of normally toxic abiotic chemical species such as H_2S, is responsible for the persistence of vent communities in the presence of high densities of predatory fishes and crabs. However, Hay et al.[35] have investigated the feeding deterrent effects of H_2S-rich blood from tubeworms and chemical extracts from unpalatable deep-sea vent organism tissues on readily accessible shallow-water generalist feeders (two crab and two fish species). Remarkably, none of the predators were deterred from feeding by the presence of H_2S in the offered food. In contrast, food containing chemical extracts from select tissues of certain polychaetes and bivalves was not accepted. Of 12 deep-sea vent species investigated, five possessed tissues that deterred feeding (*Riftia pachyptila*, *Lamellibrachia luymesi*, *Seepiophila jonesi*, *Archinome rosacea*, and *Calyptogena magnifica*). This chemical ecological study provides strong support for the use of natural product chemical defenses by deep-sea vent organisms.

Collection and Cultivation of Deep-Sea Vent Organisms

Analytical tools for molecular structure elucidation have progressed to permit routine characterization of microgram quantities of pure, unprecedented natural products. However, the characterization of new chemotypes from complex extract mixtures that also comprise a substantial mass of inorganic contaminants still requires significant biomass of source organisms. Ideally, field collections of deep-sea vent invertebrates with their associated symbionts and collectable microbial mats may need to be one-half to several liters in volume, with the possibility of re-collection, and laboratory cultivation of microorganisms of interest is highly desirable. Therefore, the logistics and potential yields of deep-sea collections are critical considerations, together with the observation that to date most hydrothermal vent microorganisms are extremely resistant to routine cultivation.

Deep-water collections can be made in a nonselective sense by dredging, trawling, and coring, which in turn damages a significant portion of nontargeted benthic communities and structures. However, advances in deep-sea submersible technology over the last 30 years have made nearly every niche of the deep ocean accessible.[100] Deep-sea submersibles impose minimal environmental impact while allowing important ecological observations and biological collections. However, the costs incurred for only 4−5 h of collection time precludes their extensive use in routine, noncollaborative collection operations.[100,101] Thus, sample collections from hydrothermal vents and cold seeps are conducted exclusively by scientific institutions that operate manned submersibles (Human Occupied Vehicles, HOV), remote operated vehicles (ROVs), or autonomous underwater vehicles (AUVs). As noted above, the most studied hydrothermal systems are in the eastern Pacific and the north-central Atlantic Oceans.[18] This is mostly due to their proximity to the countries primarily involved in deep-sea hydrothermal vent research. Table 1 lists some of the institutions and their associated submersibles with maximum depth. Access to collections of hydrothermal vent samples may occur through research collaboration with these institutions: direct participation in research cruises carrying out submersible operations yields good bulk of field-collected material in our experience. Alternately, national culture collections are a source of microbial vent samples. For example, JAMSTEC promotes collaborative efforts with industry through their Cooperative Research Project for Extremophiles program.[102] Similarly, the Brittany Microbe Culture Collection (BMCC) allows academic and industrial access to over 1300 microorganisms isolated from deep-sea hydrothermal vents by Ifremer.[103] In the United States, only the American Type Culture Collection (ATCC) publicly lists hydrothermal deep vent microorganisms available for purchase, although a small collection with

Table 1. Scientific Institutions that Operate Deep-Sea Submersibles[20,132]

institution	submersible	maximum depth (m)
Australia's Commonwealth Scientific Industrial and Research Organization (CSIRO)	AUV ABE	2500
The Canadian Scientific Submersible Facility	ROV ROPOS	5000
Florida Atlantic University's Harbor Branch Oceanographic Institution (HBOI)	HOV Johnson-Sea-Link I and II	1000
The Hawaii Undersea Research Laboratory (HURL)	HOV Pisces IV and V	2000
Institut Français De recherche pour l'Exploitation de la Mer (Ifremer)	HOV Nautile	6000
	ROV Victor	6000
The Japan Marine Science and Technology Center (JAMSTEC)	HOV Shinkai-6500	6500
	ROV Kaiko	11 000 (lost at sea 2003)
	ROV Kaiko 7000	7000
The Monterey Bay Aquarium Research Institute (MBARI)	ROV Tiburon	4000
PP Shirshov Institute of Oceanology, Russian Academy of Sciences	HOV Mir I and II	6000
Woods Hole Oceanographic Institute (WHOI)	HOV Alvin	4500
Woods Hole Oceanographic Institute (WHOI)	ROV Jason (II)	6500
Woods Hole Oceanographic Institute (WHOI)	Hybrid ROV Nereus	11 000

only 22 microorganisms is currently listed.[104] This may be a reflection of the difficulties in obtaining pure cultures of these extremophiles consistent with ATCC standards or the result of undisclosed collections due to agreements between researchers and private corporations.

Cultivation of microorganisms was central to methods used in early studies of microbial communities to determine community diversity, biomass, and production rates.[105] However, most hydrothermal vent microorganisms are extremely resistant to cultivation, which might be expected considering the extreme environments they inhabit.[64] Cultivation strategies utilizing various *in situ* colonization devices including vent cap chambers,[106] pumice-filled stainless-steel pipes,[107] titanium-mesh catheters,[108] and titanium-sheathed thermocouple arrays[109] showed moderate success in culturing some of these microbes in their natural environment for the study of *in situ* physiological expression (see Figure 2 for example studies).[110] Advances in laboratory cultivation have allowed fairly accurate replications of temperature, nutrient composition, and pressure, which have greatly increased the diversity of cultured microbes from previously "uncultivated" microorganisms.[63,111,112] Considerable effort has been applied to the large-scale cultivation of hyperthermophilic anaerobes to investigate their potential biotechnological applications.[113] Numerous biotechnology companies are actively involved in product development from thermophilic vent organisms. These biotechnological interests have focused mainly on the use of whole cells, for example, sulfate-reducing bacteria in waste management processes,[114] and also the development of new enzymes[20] and exopolysaccharides[115] to improve agriculture, biotechnology, cosmetics, pharmaceutics, and even bone healing.[116,117] In contrast, there are few reported culture efforts of likely small molecule natural product-producing microbes (e.g., Actinobacteria). Researchers in the Marine Drug Discovery Program at HBOI have isolated and cultured over 11 000 marine heterotrophic bacteria and fungi, both free-living and from invertebrate filter feeders. The Harbor Branch Marine Microbe Database[118] provides public access to detailed descriptions of microorganisms associated with deeper water (>35 m) marine invertebrates, including rRNA-based taxonomy, geographic source, depth, GenBank accession number, images, and culture and cell characteristics.[119,120] There is also a focus on laboratory cultivation of deep vent microbes at the Center for Marine Biotechnology at Rutgers University, where they have developed laboratory techniques to culture tubeworms together with their symbiotic bacteria.[121] Other successes in laboratory culture of potential natural product-producing microorganisms include the isolation of 38 actinomycetes from the Mariana Trench sediments (using marine agar and culture media selective for actinomycetes).[122] These bacteria were assigned to the *Dermacoccus, Kocuria, Micromonospora, Streptomyces, Tsukamurella*, and *Williamsia* genera based on 16S rRNA analysis. Furthermore, nonribosomal peptide synthetase (NRPS) genes were detected in more than half of the isolates, and type I polyketide synthases (PKS-I) were identified in five of the 38 strains.

Summary and Conclusions

Terrestrial microorganisms (fungi and bacteria) have had a major impact on the development of antimicrobial and antitumor compounds since the original discovery of penicillin in 1929.[123] This should be expected considering that the total global estimate of prokaryotes (archaea and bacteria) within the terrestrial subsurface (0−10 m) is 3.0×10^{29}. In comparison, deep-ocean subsurface sediments (0−10 m) are estimated to contain 6.6×10^{29} marine prokaryote cells.[104] Yet, until recently the deep ocean has been largely ignored as a source of new biologically active natural products. Importantly, much of Earth is covered by deep-marine sediments that dilute these numbers in terms of cells per total area. This is significant given the costs of sampling in the deep ocean (e.g., a 30-day expedition cruise costs roughly US $1 million with average daily operating costs of about US $30 000)[124] versus terrestrial and shallow-marine sampling costs. Thus, a more successful sampling design, in terms of increasing the likelihood of collecting a larger biomass and potentially more diverse community, should look at hydrothermal vent communities of the deep sea. Notably, decreasing numbers of microorganisms with depth to almost undetectable levels are observed in deep-ocean cold sediment cores.[125] In contrast, hydrothermally active sediments from the Guaymas Basin (Gulf of California) show high abundance and diversity of bacteria and archaea.[73] Although many new and diverse 16S rRNA sequences have been described from various hydrothermal environments, some of these communities may contain only a few dominant phylogenetic groups. However, Sogin and colleagues have shown that new molecular approaches aimed at defining the "rare biosphere", which is typically masked by conventional molecular techniques, show an even greater diversity than previously estimated by 16S rRNA analysis.[67,71] Furthermore, the impact of the geography and geological setting of hydrothermal vents on their biota implies even further untapped biological diversity awaiting discovery from the vast unexplored volcanic regions at more remote locations, which are currently beyond the manageable cost and logistics of deep-sea vent explorations. Thus, biodiscovery within these environments appears still to be in its infancy, as the full extent of the biological diversity present has yet to be realized.

Beyond a direct extrapolation of biological diversity to chemical diversity, unprecedented secondary metabolic pathways should be associated with the fundamentally different primary metabolism of these organisms, which is supported by altered protein and lipid compositions, conformations, and binding activities.[9] The presence of chemical defenses in some deep-sea vent invertebrates is implied by the feeding-deterrent assays reported by Hay et al.[35] Additionally, anaerobic cycling of carbon often requires close associations of interdependent microorganisms, and these microbial interactions may be supported by an efficient communication network of small signaling molecules of potential utility for human health applications. New, more advanced deep-sea research technology and molecular techniques similar to those already employed at Verenium

(formerly Diversa Corporation) and other research institutions are aimed at screening a more inclusive genetic assembly and may permit a molecular genomics approach to accelerate natural product discoveries from deep-sea vent environments. The source material for this natural products chemistry appears to be accessible from collections of bulk field samples using deep-sea submersibles and from laboratory isolation and cultivation of some microorganisms. No assessment of the potential for discovery of new chemical templates can be made based on the sole reports to date of the loihichelins and ammonificins from deep-sea vent organisms, and indeed it is too early to designate deep-sea vents as natural products "hot spots". However, the burgeoning evidence of microbial diversity and concomitant species competition and syntropy/symbiosis, in tandem with the potential to integrate biological sampling for natural products research with ongoing deep-sea vent explorations, warrants concerted natural products investigations of deep-sea hydrothermal vent and cold-seep environments.

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