

Microbe–metal interactions in marine hydrothermal environments

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Marine hydrothermal microorganisms respond rapidly to changes in the concentrations and availability of metals within their environment. Hyperthermophilic archaea appear to possess novel mechanisms for metal detoxification, dissimilatory metal reduction and metal assimilation that may be absent in their mesophilic and bacterial counterparts. For example, tungsten was found in high concentrations in a hydrothermal sulfide deposit where hyperthermophiles were also most abundant, consistent with the unique requirement of these organisms for this element. Furthermore, newly isolated genera of iron-reducing hyperthermophiles expand the scope of carbon cycling in hydrothermal environments. The advent of genome sequences and new molecular techniques will facilitate our further understanding of microbe–mineral interactions in these environments.

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Abbreviations

BAB back-arc basin
MOSC mid-ocean spreading center
SMV shallow marine vent

Introduction

One of the most metal-rich biotopes known are hydrothermal environments where geothermally heated water reacts with its host rock forming fluids that nourish a diverse array of geothermally dependent microorganisms. These sites include deep-sea hydrothermal vents along mid-ocean spreading centers (MOSCs) and terrestrial hot springs, as well as lesser-known shallow marine vents (SMVs) and marine back-arc basins (BABs) (i.e. zones of oceanic lithosphere subduction). Little is known about how microorganisms from marine hydrothermal environments interact with metals *in situ*, but their interactions are generally described in one of three ways: the metals are toxic and illicit a response; they are oxidized or

reduced to conserve energy in dissimilatory reactions; or they are taken up and utilized in assimilatory reactions. This review focuses on recent findings involving all three of these metal–microbe interactions and their significance with respect to the physiology and ecology of microbes in hydrothermal environments.

Fluid chemistries of marine hydrothermal environments

Hydrothermal fluids differ from seawater (Table 1) because of a series of seawater–host-rock chemical reactions as the fluids circulate through the seafloor [1••]. In general, cool seawater migrates downward in the crust through a fine-scale network of cracks. At temperatures below 100°C, alkalis such as K, Rb and Cs as well as rare-earth elements are stripped from seawater during mineral replacement reactions with the host pillow basalts and sheet flows. Uptake of these elements is balanced in the rocks by loss of Mg, Si and S. As temperatures reach 350–550°C, elements such as Cu, Zn, Fe, Pb and S, as well as SiO₂, are leached out of the rocks and into the circulating fluids. As the hot buoyant fluids ascend to the surface, elements such as K, Rb, Li and rare-earth elements are leached from the basaltic material at temperatures above 150°C. In most deep-sea basaltic environments, these fluids reach the seafloor at temperatures of 350–400°C and are reduced and acidic (pH 2–6). Relative to seawater, they are highly enriched in Fe, Mn, Zn, Cu, Co and As, depleted in Mg, and either enriched or depleted in Ca, Se and Mo depending on the chemical composition of the basaltic rock at the fluid–rock interfaces during their downward and upward migrations. Hydrothermal fluid may also mix with seawater within the crust, resulting in exit fluids with both seawater and hydrothermal fluid traits.

The inventory and reactivity of metal species in marine hydrothermal fluids vary both spatially and temporally due to the chemical compositions of the fluids and rocks involved, the input of allochthonous (i.e. imported from a contiguous system) material, and the temperatures and pressures of the reactions (Table 1). MOSC hydrothermal environments are found typically in lightly sedimented young rocks composed primarily of basalts and gabbros. Seawater enters a MOSC and frequently evolves into hydrothermal fluid by passing through a two-phase condition during its sub-seafloor reaction path due to the high temperatures and pressures experienced by the fluid [2]. This phase separation creates a low-chlorinity, low-metal vapour phase that is emitted from the seafloor before the release of the high-metal, low-vapour brine phase. The exception to this process involves those metals that form volatile species, such as arsenic and antimony, which are

Table 1

Metals in various types of hydrothermal fluids.

Metal (conc.)	Biological response	MOSCs [25*,34]	BABs [25*,34]	SMVs [4]	Seawater [35]
Fe (mM)	D,A	0.01–1362	1.2–2.9	0–0.9	0.000061
Mn (mM)	D	0.1–1150	5.8–7.1	0–0.3	0.000007
Ca (mM)	A	2–109	28–41	0.5–18	10
Mg (mM)	A	0–53	0–53	0.3–67	53
Zn (μ M)	T,A	0.7–780	1200–3100	0–17	0.076
Cu (μ M)	T,A	0.1–21	15–35	0.02–1.9	0.014
Ni (nM)	T,A	nd	nd	2–586	112
Co (nM)	T,A	35–1430	3800–7000	2–287	7
As (nM)	T,D	30–1074	6000–11 000	8–1789	35
Se (nM)	T,D,A	0.6–103	nd	10–456	11
Mo (nM)	A	1–33	nd	3–4200	104
W (nM)	A	2	nd	0.5–315	0.5

Abbreviations: A, assimilatory reaction; D, dissimilatory reaction; nd, no available data; T, toxicity response.

elevated in vapour-phase fluids [3]. Phase separation significantly affects the kinds of microorganisms found associated with hydrothermal environments as well as the level of microbial activity within the environment [2]. By contrast, BAB hydrothermal environments are found in sediment-covered acidic rocks [3] and the high acidity of BAB fluids results in much higher metal concentrations than are found in MOSC or SMV hydrothermal fluids (Table 1). On the other hand, the metal concentrations within SMV fluids are higher than in seawater but are not as elevated as those found at MOSC and BAB sites (Table 1) due to the absence of phase separation and to differences in rock composition [4].

Microorganisms found in marine hydrothermal environments

The microorganisms associated with marine hydrothermal environments are generally described in one of four ways on the basis of their habitat: those in the pore spaces and cracks below the surface; those on the outer surface of the sulfide deposits, typically as mats; those on and within invertebrates often as symbionts; and those within the plume of hydrothermal fluid in the overlying seawater [1]. The latter three groups are generally mesophilic (i.e. optimal growth 20–45°C) aerobic bacteria, some of which oxidize Fe and Mn for their respiration. Subsurface microbes are primarily aerobic bacteria at lower temperatures and shift to mostly anaerobic archaea as temperatures reach 100°C (i.e. hyperthermophiles, optimal growth >80°C). Bacteria and archaea are unicellular organisms that lack a nucleus making them distinct from eukaryotes. However, these two domains of life are distinguished by their ribosomal nucleotide sequences [5] and the presence of ester-linked lipids in bacteria and ether-linked lipids in archaea.

Toxicity responses

Microorganisms respond to the presence of potentially toxic metal ions through several intrinsic mechanisms to regulate intracellular concentrations [6,7]:

1. Exopolysaccharide production.
2. Detoxification through specific efflux systems.
3. Metal sequestration by specific mineral ion binding components.
4. Tight coupling between membrane transport metal efflux proteins and ATPases.
5. Enzymatic transformation converting more toxic to less toxic or less available metal-ion species.

In this regard, the archaea that are found in hydrothermal vent systems appear to have unusual responses. For example, 16 anaerobic hyperthermophilic sulfur-reducing organisms, 10 of the archaeal order *Thermococcales* and six of the bacterial order *Thermotogales*, were isolated from a BAB site to test their susceptibility to heavy metals [8]. The *Thermococcales* could withstand Cd and Zn concentrations that were 100–1000 fold and 10–100 fold higher, respectively, than those withstood by the *Thermotogales* as well as the moderately thermophilic *Bacillus* strains isolated from the same site. The significantly higher metal resistance in the *Thermococcales* suggests that hyperthermophilic archaea possess one or more novel mechanisms for dealing with the toxic metals that are found within hydrothermal environments, mechanisms that are lacking or underdeveloped in their bacterial counterparts. Indeed, complete genome sequences of several members of the *Thermococcales* are now available and close to half of their putative genes are of unknown function, many of which are unique to archaea [9*]. These unique unknown genes may encode for these putative novel mechanisms.

Tolerance to toxic metal ions in hydrothermal vents is not limited to hyperthermophilic organisms. One study involved a moderately thermophilic *Bacillus* strain, which grows at 65°C, collected from the same BAB site. Resistance to Cd was higher when cultures were previously grown in the presence of the metal, suggesting that this acclimation was induced [8]. Similarly, 10 tellurite- and selenite-reducing strains of *Pseudoalteromonas* spp. were

isolated at 30°C from a MOSC site in the north-eastern Pacific Ocean [10•]. The lowest concentrations of K_2TeO_3 and Na_2SeO_3 that inhibited growth were $1500 \mu\text{g ml}^{-1}$ and $6000 \mu\text{g ml}^{-1}$, respectively, which are comparable to the highest levels of tellurite and selenite resistance known in bacteria [10•]. The mechanisms of Te or Se resistance or the induced Cd tolerance are poorly understood. As with the hyperthermophiles, there may be novel physiological adaptations among mesophilic bacteria to hydrothermal ecosystems or, more specifically, to their unique inventory and speciation of metals.

Dissimilatory reactions

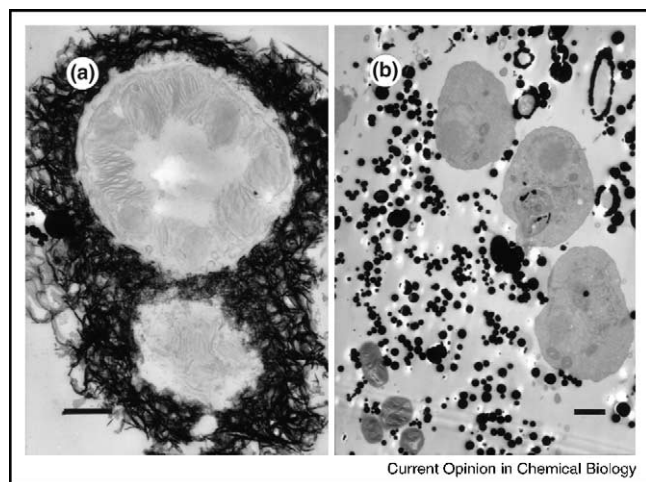
In dissimilatory reactions, the metal ion is used as an electron donor or acceptor during respiration and is typically not incorporated into the cell. The use of iron now appears to be a common metal-dependent respiratory mode. In fact, organisms with this ability appear to be readily able to colonize hydrothermal environments. For example, following the 1996 volcanic eruption at a MOSC site off the Oregon coast, phase separation of hydrothermal fluids led to extensive microbial growth within the seafloor [11]. Remarkably, there was no evidence of hydrothermal venting or extensive microbial communities at the site before the eruption. Nevertheless, thick (>10 cm) mats of nearly exclusive iron-oxidizing bacteria were associated with the more-evolved metal-rich brine fluids (Figure 1a). In addition, copious biogenic-sulfur

flocculent material, high concentrations of morphologically diverse microorganisms, and primarily sulfur-oxidizing bacterial mats, but few iron-oxidizers, were associated with metal-poor fluids found venting 30 km away as a result of the same eruption (Figure 1b) [11]. Iron-oxidizing bacteria also were ubiquitous around hydrothermal venting at Loihi Seamount, Hawaii and were major inhabitants of newly formed vents following a 1996 eruption on the seamount [12•]. A conservative estimate suggests that up to 60% of the iron oxide deposition around the Loihi vents is due to iron-oxidizing bacteria, which is comparable to the iron oxidizing activity measured in freshwater microcosm experiments. These examples nicely illustrate the ability of previously quiescent microbes to sense and mount a rapid response to the sudden emergence of hydrothermal fluids of a specific chemical composition, again by as-yet unknown mechanisms.

Some hyperthermophiles appear to be highly adapted to toxic metal ions (or metalloids). Arsenate and selenate are generally toxic to microorganisms; however, the hyperthermophilic crenarchaeon *Pyrobaculum arsenaticum*, which was isolated from a hot, freshwater spring near Naples, Italy, grows on hydrogen and carbon dioxide as its energy and carbon sources using 10 mM arsenate or 50 mM selenate as the electron acceptor [13]. *Pyrobaculum islandicum* is capable of oxidizing H_2 or components of peptone or yeast extract and reducing various metals such as Fe(III), U(VI), Tc(VII), Cr(VI), Co(III) and Mn(IV) at 100°C but the organism did not reduce As(V) or Se(VI) [14]. *P. islandicum* also oxidized humic compounds and the humic analogue anthraquinone-2,6-disulfonate (AQDS). These reactions greatly accelerated the rate of reduction of poorly crystalline Fe(III) oxide, suggesting that humic compounds may serve as an extracellular electron shuttle for metal reduction [15]. Iron reduction in *P. islandicum* occurs by a mechanism that differs from the NADH- and cytochrome *c*-dependent process that occurs in mesophilic iron reducers such as species of *Shewanella* and *Geobacter*. *P. islandicum* does not contain *c*-type cytochromes and NADPH was better than NADH ($K_m = 0.04$ and 3.33 mM, respectively) as an electron donor for Fe(III) reductase activity [16••].

It has been suggested that iron reduction is widespread among hyperthermophiles and may represent a primitive metabolism [17]. For example, the number of culturable thermophilic (52–90°C) iron reducers in MOSC sulfide material was estimated to range from 10 to 10^7 cells cm^{-3} [18•]. A hyperthermophilic iron reducer belonging to the genus *Thermococcus* was purified from a vent sample [18•] and thermophilic iron reducers belonging to the genera *Thermoanaerobacter*, *Thermotoga* and *Thermococcus* were also isolated from a 60–84°C deep subsurface petroleum reservoir in western Siberia [19]. These organisms therefore appear to be ubiquitous in geothermal environments,

Figure 1



Thin-section electron micrographs of bacterial mat material collected from the CoAxial segment MOSC site in the north-eastern Pacific Ocean three months after a volcanic eruption had occurred producing new hydrothermal venting (Holden JF, Baross JA, unpublished data). The samples were from (a) high metal and brine, low-vapour containing fluids and (b) low metal and brine, high vapour containing hydrothermal fluids that were 30 km apart but created from the same eruption. Iron oxidizers that deposit iron onto their sheaths are seen in (a) whereas, in (b), putative methane-oxidizing bacteria (lower left), protozoa and sulfur oxidizers that deposit elemental sulfur onto their sheaths (top right) are seen. Scale bars: (a), 0.5 μm ; (b), 1 μm .

although it is not clear if any of them are able to gain energy from the process of iron reduction, or whether these fermentative organisms simply use it as an electron acceptor. However, there are some hyperthermophiles that use Fe(III) in respiratory processes, and these include the novel dissimilatory iron reducers *Geoglobus ahangari* and *Geothermobacterium ferrireducens*, which were isolated from the Guaymas Basin MOSC site and Yellowstone National Park, respectively [20**,21**]. Moreover, the ability of *G. ahangari* and *Ferroglobus placidus* to couple the oxidation of acetate to Fe(III) reduction in essence completed the carbon cycle at hyperthermophilic temperatures. Thus, CO₂ fixation by autotrophs and CO₂ and acetate production by heterotrophs could be linked by the conversion of acetate to CO₂ [22**]. Reductive gold precipitation (Au(III) to Au(0)) was also observed in four of seven hyperthermophile species tested (*P. islandicum*, *G. ahangari*, *Pyrococcus furiosus* and *Thermotoga maritima*, but not *Pyrobaculum aerophilum*, *Archaeoglobus fulgidus* or *F. placidus*) with H₂ as the electron donor and deposition of the gold on the exterior surface of the cells [23*]. However, the physiological significance of such reactions is unclear since neither *P. furiosus* nor *T. maritima* are known to oxidize H₂.

Assimilatory reactions

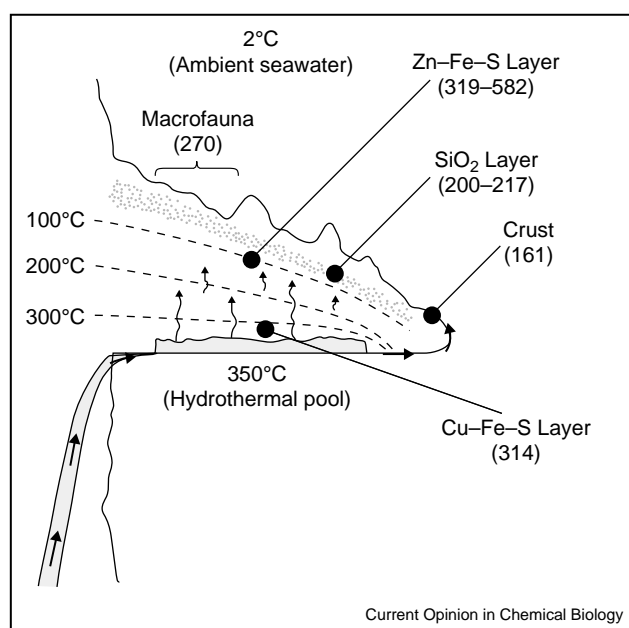
With assimilatory reactions, the metal that is oxidized or reduced is incorporated into cellular material as a cofactor in enzymes (e.g. in oxidoreductases). Unfortunately, not much is known about the assimilation of metals by microorganisms in hydrothermal environments, with two notable exceptions. The first is iron assimilation in *Archaeoglobus fulgidus*, which appears to occur via a novel cytoplasmic ferric reductase [24,25*]. This protein is a 40 kDa homodimer that uses FMN as a cofactor and reduces Fe³⁺–EDTA using both NADH and NADPH as electron donors. Its catalytic activity resembles bacterial and eukaryotic assimilatory ferric reductases, but its high cellular abundance (~0.75% of the total soluble protein) suggests a possible catabolic role for this enzyme as the terminal electron acceptor [25*].

The second notable example of metal assimilation involves the role of the metal tungsten (W). In general, studies with a wide range of microorganisms indicate that W is typically preferred over molybdenum (Mo) with increasing growth temperatures above 60°C or so. These metals are used in the active sites of a range of oxidoreductase-type enzymes and mesophilic organisms almost exclusively utilize Mo. The preference for W at the higher temperatures appears to be due both to the differences in the chemical properties of W and Mo as well as their availability in hydrothermal environments [26*]. With both metals, the active site of the enzymes contains a mononuclear centre that is coordinated by two or four dithiolene sulfur atoms from one or two pterin molecules that frequently function in conjunction with a

single [Fe₄–S₄] cluster [26*]. W complexes appear to display greater thermal stability and, although they are also much more O₂-sensitive than Mo complexes, the W-enzymes are typically found in obligate anaerobes. It was thought that W was used exclusively at temperatures near 100°C [27], but *P. aerophilum* was shown to use Mo as well as W [28]. By contrast, *P. furiosus* appears incapable of utilizing Mo even when cells are limited for W [29].

There also seems to be a correlation between the amounts of W present and the environments where hyperthermophiles are found. In such hydrothermal ecosystems, sulfide deposits form when metal–sulfide-rich hydrothermal fluids react with oxidic seawater and these deposits form a habitat for hyperthermophiles. One rather unusual type of hydrothermal deposit is known as a flange and consists of a horizontal outcrop of sulfidic rock that traps hot buoyant hydrothermal fluid on the underside of the outcrop (Figure 2) [30]. The hot fluid ‘spills’ over the edge of the flange and also percolates through the porous rock creating a temperature gradient from 200–350°C below the outcrop to 1–2°C (ambient seawater temperature) above it. An analysis for W within one such flange revealed that the concentrations of W were highest in the soft porous Zn–Fe–S layer composed of marcasite, wurtzite and sphalerite (Adams MWW, Baross J, unpublished data) that precipitates from hydrothermal fluids at temperatures below 250°C (Figure 2) [31]. A vertical profile lipid analysis of a similar flange revealed

Figure 2



Tungsten concentrations (mmol g⁻¹) in various regions of a hydrothermal sulfide deposit, or a flange, collected from the Endeavour segment MOSC site in the north-eastern Pacific Ocean (Adams MWW, Baross J, unpublished data).

that the highest concentrations of ether-linked lipids, which are only produced by archaea, was in the Zn–Fe–S deposition zone where bacterial lipid concentrations were significantly lower [32]. Hyperthermophilic archaea rather than hyperthermophilic bacteria are readily purified from this Zn–Fe–S sulfide material. Therefore, this correlation between W and hyperthermophile concentrations begs the question of whether the high W concentrations are due to geochemical reactions that then attract hyperthermophiles or whether the W is accumulated and deposited in this layer by the hyperthermophiles, which may have a high affinity for W in hydrothermal fluids.

Conclusions

Although microorganisms found in hydrothermal environments must interact with toxic and non-toxic metals in much the same way that other microorganisms interact with metals in their environments, there appear to be exciting and novel differences regarding the details of interactions within this environment. It appears that hyperthermophilic archaea may be more resilient to toxic metals than their bacterial counterparts and may catalyse dissimilatory metal reduction in a manner not found previously. Furthermore, hyperthermophiles have a preference for W over Mo, although it is not clear whether this is primarily a consequence of W availability or the chemical properties of the element with regard to the catalysis of low potential reactions at high temperatures. Insights into these potentially novel processes may be elucidated using functional genomic approaches as the genomes of ten hyperthermophilic archaea have been completely sequenced and annotated [33]. However, very little is known about the environmental signaling and physiological responses of microbes when they encounter various metals, particularly in hydrothermal vent environments. In addition, there is little information on the properties of natural assemblages of microorganisms, particularly in biofilms. Coupled with the dearth of data from hydrothermal fluid metal analyses, especially from BAB and SMV environments, our basic understanding of microbe–metal interactions in hydrothermal environments is still extremely limited. Analyses of these environments that examine spatial and temporal variations of metals and microbes in fluids are clearly needed, and these need to be related to physiological studies on natural microbial communities. It is hoped that the reports referred to in this article will stimulate new experimental approaches to studying metals in hot environments, about which little is really known.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Kelley DS, Baross JA, Delaney JR: **Volcanoes, fluids and life at mid-ocean ridge spreading centers**. *Annu Rev Earth Planet Sci* 2002, **30**:385-391.
This is a very thorough review of the geochemistry, geophysics and geology of hydrothermal systems along MOSCs. What is known about microbial life associated with these environments and their implications regarding the deep subsurface biosphere, the origin of life and the potential for life on other solar bodies are discussed at length.
 2. Butterfield DA, Jonasson IR, Massoth GJ, Feely RA, Roe KK, Embley RE, Holden JF, McDuff RE, Lilley MD, Delaney JR: **Seafloor eruptions and evolution of hydrothermal fluid chemistry**. *Phil Trans R Soc Lond A* 1997, **355**:369-386.
 3. Douville E, Charlou J-L, Donval J-P, Hureau D, Appriou P: **As and Sb behaviour in fluids from various deep-sea hydrothermal systems**. *Earth Planet Sci* 1999, **328**:97-104.
 4. Aiuppa A, Dongarrà G, Capasso G, Allard P: **Trace elements in the thermal groundwaters of Vulcano Island (Sicily)**. *J Volcanol Geotherm Res* 2000, **98**:189-207.
 5. Pace NR: **A molecular view of microbial diversity and the biosphere**. *Science* 1997, **276**:734-740.
 6. Gadd GM: **Molecular biology and biotechnology of microbial interactions with organic and inorganic heavy metal compounds**. In *Molecular Biology and Biotechnology of Extremophiles*. Edited by Herbert RA, Sharp RJ. New York: Chapman and Hall; 1992:225-257.
 7. Silver S, Phung LT: **Bacterial heavy metal resistance: new surprises**. *Annu Rev Microbiol* 1996, **50**:753-789.
 8. Llanos J, Capasso C, Parisi E, Prieur D, Jeanthon C: **Susceptibility to heavy metals and cadmium accumulation in aerobic and anaerobic thermophilic microorganisms isolated from deep-sea hydrothermal vents**. *Curr Microbiol* 2000, **41**:201-205.
 9. Holden J, Adams MWW: **Unique aspects of the hyperthermophile proteome**. In *Encyclopedia of Life Support Systems: Extremophiles*, Vol. 6.73. Edited by Gerday C. Paris: UNESCO; 2003, in press.
The first comprehensive review of proteins characterized from a subset of hyperthermophiles. It describes those proteins that are unique to hyperthermophiles and archaea and predicts what proportion of the proteins within hyperthermophilic archaea is found only within this domain.
 10. Rathgeber C, Yurkova N, Stackebrandt E, Beatty JT, Yurkov V: **Isolation of tellurite- and selenite-resistant bacteria from hydrothermal vents of the Juan de Fuca Ridge in the Pacific Ocean**. *Appl Environ Microbiol* 2002, **68**:4613-4622.
The first report of tellurite and selenite resistance from a hydrothermal environment.
 11. Juniper SK, Martineau P, Sarrazin J, Gelinat Y: **Microbial-mineral floc associated with nascent hydrothermal activity on CoAxial segment, Juan de Fuca Ridge**. *Geophys Res Lett* 1995, **22**:179-182.
 12. Emerson D, Moyer CL: **Neutrophilic Fe-oxidizing bacteria are abundant at the Loihi Seamount hydrothermal vents and play a major role in Fe oxide deposition**. *Appl Environ Microbiol* 2002, **68**:3085-3093.
A thorough report of the extent and morphological characteristics of iron oxidizing bacteria at a deep-sea hydrothermal vent site near Hawaii.
 13. Huber R, Sacher M, Vollmann A, Huber H, Rose D: **Respiration of arsenate and selenate by hyperthermophilic archaea**. *Syst Appl Microbiol* 2000, **23**:305-314.
 14. Kashefi K, Lovley DR: **Reduction of Fe(III), Mn(IV), and toxic metals at 100 °C by *Pyrobaculum islandicum***. *Appl Environ Microbiol* 2000, **66**:1050-1056.
 15. Lovley DR, Kashefi K, Vargas M, Tor JM, Blunt-Harris EL: **Reduction of humic substances and Fe(III) by hyperthermophilic microorganisms**. *Chem Geol* 2000, **169**:289-298.

16. Childers SE, Lovley DR: **Differences in Fe(III) reduction in the hyperthermophilic archaeon, *Pyrobaculum islandicum*, versus mesophilic Fe(III)-reducing bacteria.** *FEMS Microbiol Lett* 2001, **195**:253-258.
- This research shows that the enzymatic mechanism for iron reduction in this hyperthermophile is unique relative to that found in mesophilic bacteria on the basis of the absence of cytochrome *c* and the enzyme's preference for NADPH over NADH.
17. Vargas M, Kashefi K, Blunt-Harris EL, Lovley DR: **Microbiological evidence for Fe(III) reduction on early earth.** *Nature* 1998, **395**:65-67.
18. Slobodkin A, Campbell B, Cary SC, Bonch-Osmolovskaya E, Jeanthon C: **Evidence for the presence of thermophilic Fe(III)-reducing microorganisms in deep-sea hydrothermal vents at 13° N (East Pacific Rise).** *FEMS Microbiol Ecol* 2001, **36**:235-243.
- This is the first work to specifically target, isolate and phylogenetically categorize thermophilic and hyperthermophilic iron reducers from a deep-sea hydrothermal environment.
19. Slobodkin AI, Jeanthon C, L'Haridon S, Nazina T, Miroshnichenko M, Bonch-Osmolovskaya E: **Dissimilatory reduction of Fe(III) by thermophilic bacteria and archaea in deep subsurface petroleum reservoirs of western Siberia.** *Curr Microbiol* 1999, **39**:99-102.
20. Kashefi K, Tor JM, Holmes DE, Gaw Van Praagh CV, Reysenbach A-L, Lovley DR: ***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.** *Int J Syst Evol Microbiol* 2002, **52**:719-728.
- See annotation to [21**].
21. Kashefi K, Holmes DE, Reysenbach A-L, Lovley DR: **Use of Fe(III) as an electron acceptor to recover previously uncultured hyperthermophiles: isolation and characterization of *Geothermobacterium ferrireducens* gen. nov., sp. nov.** *Appl Environ Microbiol* 2002, **68**:1735-1742.
- These two papers [20**,21**] are the first detailed descriptions of hyperthermophilic iron reducers and demonstrate that these organisms include archaea and bacteria and are found in marine and fresh water environments, all of which suggests that microbial iron reduction may be an ancient and wide-spread trait.
22. Tor JM, Kashefi K, Lovley DR: **Acetate oxidation coupled to Fe(III) reduction in hyperthermophilic microorganisms.** *Appl Environ Microbiol* 2001, **67**:1363-1365.
- This work largely closes the hyperthermophilic carbon cycle that begins with CO₂ fixation by methogens and other autotrophs and continues with CO₂ and acetate production by the heterotrophs. It demonstrates that hyperthermophilic iron reducers can oxidize acetate to CO₂.
23. Kashefi K, Tor JM, Nevin KP, Lovley DR: **Reductive precipitation of gold by dissimilatory Fe(III)-reducing Bacteria and Archaea.** *Appl Environ Microbiol* 2001, **67**:3275-3279.
- The work demonstrates that some hyperthermophiles, but not all, are capable of reducing gold and depositing it on the cell's exterior surface.
24. Vadas A, Monbouquette HG, Johnson E, Schröder I: **Identification and characterization of a novel ferric reductase from the hyperthermophilic archaeon *Archaeoglobus fulgidus*.** *J Biol Chem* 1999, **274**:36715-36721.
25. Chiu H-J, Johnson E, Schröder I, Rees DC: **Crystal structures of a novel ferric reductase from the hyperthermophilic archaeon *Archaeoglobus fulgidus* and its complex with NADP⁺.** *Structure* 2001, **9**:311-319.
- This work shows the first crystal structure of a hyperthermophilic iron reductase as well as those of enzyme-FMN and enzyme-FMN-NADP⁺ intermediates.
26. Roy R, Adams MWW: **Tungsten-dependent aldehyde oxidoreductase: a new family of enzymes containing the pterin cofactor.** *Metal Ions Biol Systems* 2002, **39**:673-697.
- The latest and most comprehensive review of hyperthermophilic, tungsten-containing aldehyde oxidoreductase biochemistry, phylogeny and structure.
27. Kletzin A, Adams MWW: **Tungsten in biological systems.** *FEMS Microbiol Rev* 1996, **18**:5-63.
28. Afshar S, Kim C, Monbouquette HG, Schröder I: **Effect of tungstate on nitrate reduction by the hyperthermophilic archaeon *Pyrobaculum aerophilum*.** *Appl Environ Microbiol* 1998, **64**:3004-3008.
29. Mukund S, Adams MWW: **Molybdenum and vanadium do not replace tungsten in the catalytically active forms of the three tungstoenzymes in the hyperthermophilic archaeon *Pyrococcus furiosus*.** *J Bacteriol* 1996, **178**:163-167.
30. Delaney JR, Robigou V, McDuff RE, Tivey MK: **Geology of a vigorous hydrothermal system on the Endeavour Segment, Juan de Fuca Ridge.** *J Geophys Res* 1992, **97**:19663-19682.
31. Koski RA, Jonasson IR, Kadko DC, Smith VK, Wond FL: **Compositions, growth mechanisms, and temporal relations of hydrothermal sulfide-sulfate-silica chimneys at the northern Cleft segment, Juan de Fuca Ridge.** *J Geophys Res* 1994, **99**:4813-4832.
32. Hedrick DB, Pledger RJ, White DC, Baross JA: **In situ microbial ecology of hydrothermal vent sediments.** *FEMS Microbiol Ecol* 1992, **101**:1-10.
33. National Center for Biotechnology Information (NCBI) of the NIH on URL: <http://www.ncbi.nlm.nih.gov> gives complete and annotated genome sequences of over 200 microorganisms, including 10 hyperthermophile species.
34. Von Damm KL: **Controls on the chemistry and temporal variability of seafloor hydrothermal fluids.** In *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*, Geophysical Monograph 91. Edited by Humphris SE, Zierenberg RA, Mullineaux LS, Thomson RE. Washington, DC: American Geophysical Union; 1995:222-247.
35. Turekian KK: *Oceans*. Englewood Cliffs, New Jersey: Prentice-Hall; 1968:120 pages.