Microbes in mercury-enriched geothermal springs in western North America

Authors: Gill G. Geesey, Tamar Barkay, and Sue King.

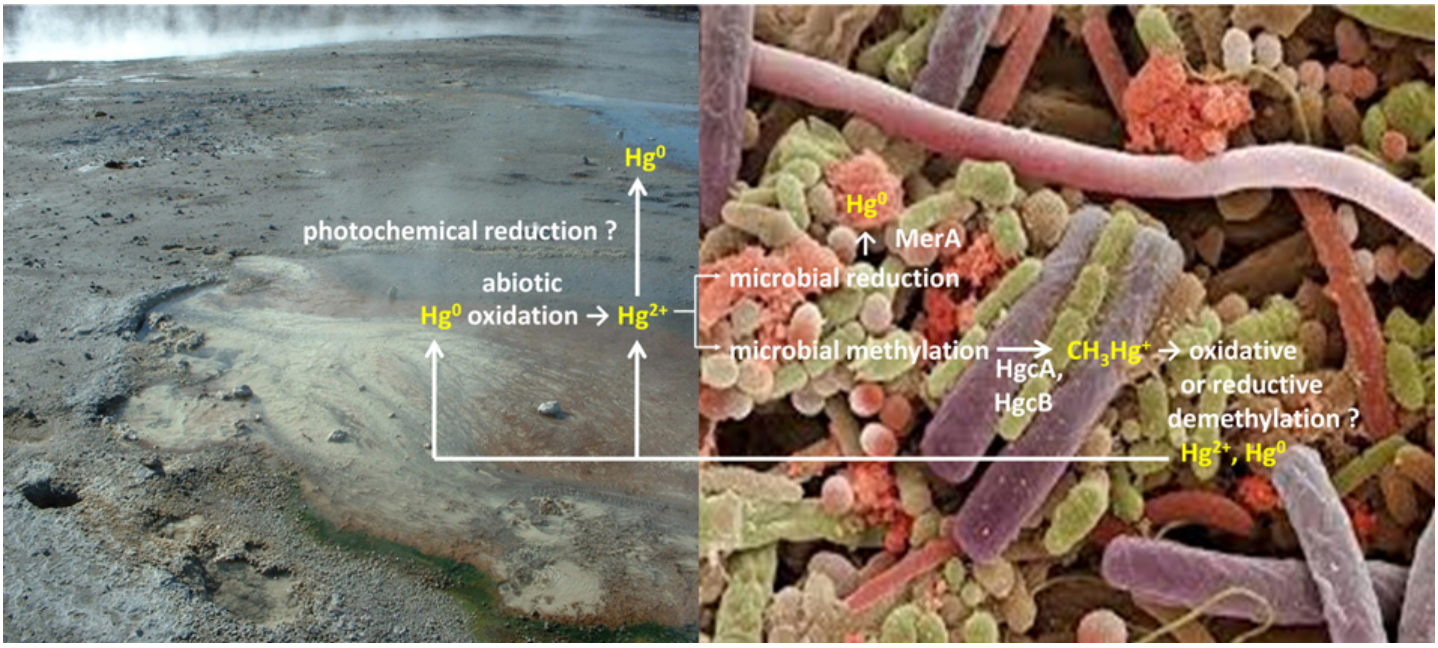
Geesey GG, Barkay T, King S "Microbes in mercury-enriched geothermal springs in western North America," Sci Total Environ, 2016 Nov 1; 569–570:321–331.

Microbes in mercury-enriched geothermal springs in western North America

Gill G. Geesey [a,*], Tamar Barkay [b], Sue King [c]

[a] Department of Microbiology and Immunology, Thermal Biology Institute, Montana State University, Bozeman, MT 59717-3520, USA
[b] Department of Biochemistry and Microbiology, Graduate Program in Ecology and Evolution, Rutgers University, New Brunswick, NJ 08901-8525, USA [c] 2908 3rd Avenue North, Great Falls, MT 59401, USA

GRAPHICAL ABSTRACT



ABSTRACT

Because geothermal environments contain mercury (Hg) from natural sources, microorganisms that evolved in these systems have likely adapted to this element. Knowledge of the interactions between microorganisms and Hg in geothermal systems may assist in understanding the long-term evolution of microbial adaptation to Hg with relevance to other environments where Hg is introduced from anthropogenic sources. A number of micro-biological studies with supporting geochemistry have been conducted in geothermal systems across western North America. Approximately 1 in 5 study sites include measurements of Hg. Of all prokaryotic taxa reported across sites with microbiological and accompanying physicochemical data, 42% have been detected at sites in which Hg was measured. Genes specifying Hg reduction and detoxification by microorganisms were detected in a number of hot springs across the region. Archaeal-like sequences, representing two crenarchaeal orders and one order each of the Euryarchaeota and Thaumarchaeota, dominated in metagenomes' MerA (the mercuric reductase protein) inventories, while bacterial homologs were mostly found in one deeply sequenced metagenome. MerA homologs were more frequently found in metagenomes of microbial communities in acidic springs than in circumneutral or high pH geothermal systems, possibly reflecting higher bioavailability of Hg under acidic conditions. MerA homologs were found in hot springs prokaryotic isolates affiliated with Bacteria and Archaea taxa. Acidic sites with high Hg concentrations contain more of Archaea than Bacteria taxa, while the reverse appears to be the case in circumneutral and high pH sites with high Hg concentrations. However, MerA was detected in only a small fraction of the Archaea and Bacteria taxa inhabiting sites containing Hg. Nev-ertheless, the presence of MerA homologs and their distribution patterns in systems, in which Hg has yet to be measured, demonstrates the potential for detoxification by Hg reduction in these geothermal systems, particularly the low pH springs that are dominated by Archaea.

HIGHLIGHTS

- Of 245 prokaryotic taxa at 271 geother-mal sites, 103 were in 56 sites with Hg.
- 46% of Archaea and 40% of Bacteria taxa were in those reported to contain Hg.
- Richness of Bacteria NArchaea across the Hg-containing sites with pH ≥ 4.5
- Homologs of Mer genes for Hg reduc-tion at higher frequency in acidic sites
- MerA homologs in Western U.S. hot springs are most like those in 4 archaeal orders.

KEYWORDS

Microorganisms
Hg resistance
Hot springs
Geochemistry, MerA, Archaea, Bacteria

1. Introduction

Western North America (NA) hosts a large number of hot springs, many of which result from the tectonic processes in this part of the world. Accordingly, many are associated with the belts of recent past or present volcanic activity. Thermal springs are also found in other areas where rocks have been faulted and intensely folded in geologically recent time. Approximately 840 geothermal areas have been identified in the western contiguous United States, not counting the >10.000 geothermal features found in Yellowstone National Park, and the additional 79 identified in Alaska (Waring, 1965). Across this geothermal landscape, temperatures range from slightly above ambient to over 100 °C (Berry et al., 1980), pH ranges from 1 to 10 (Ball et al., 2008; Thompson and DeMonge, 1996) and sulfide concentrations range from <0.3 to 5.8 mM (Dodsworth and Hedlund, 2010; Kulp et al., 2008). This wide range of environmental conditions creates niches that support diverse assemblages of prokaryotic life (Spear et al., 2006). Of the environmental parameters examined to date, temperature, pH, and sulfide vs. dissolved oxygen concentrations have been shown to be the key determinants of microbial community composition in a number of these geothermal systems (Inskeep et al., 2013; Miller et al., 2009; Purcell et al., 2007; Sharp et al., 2014).

Many geothermal systems also contain toxic metals, among them mercury (Hg) (Becker, 1888; Cobble, 1987; de Beaumont, 1847; Dreyer, 1940a; Dreyer, 1940b; White, 1955; White, 1957; White et al., 1963). Mercury in these systems exists in gaseous, liquid and solid forms. Subsurface hydrothermal fluids leach Hg from reservoir rock and transport it to the surface in either an aqueous or vapor phase (Fig. 1). The concentration and solubility of Hg in these phases is influenced by the amount of Hg in the rock as well as by fluid temperature and chemistry (Engle et al., 2006). Gaseous Hg typically exists as Hg^0 and HgS. Thermodynamic calculations indicate that aqueous ionic Hg may exist at parts per trillion concentrations, depending on conditions (Cobble, 1987; Varekamp and Buseck, 1984). Since most analyses measure only total Hg (THg) after filtration to remove suspended solids, the concentrations of the different forms of aqueous ionic Hg that exist under the various geothermal conditions are not well documented. However, it is known that sulfide and chloride concentrations and temperature are important determinants of the forms and concentrations of aqueous ionic Hg species in these systems (Glew and Hames, 1972; Krup, 1988). The concentration of THg can be as high as 2.23 mg/L in unfiltered acidic, sulfidic hot spring water as a result of the penetration of thermal plumes of cinnabar (Simbahan et al., 2005). If Hg is transported in the fluid as Hg-S or Hg-Cl, it can precipitate as cinnabar, metacinnabar or calomel, the former being the more stable under hot springs conditions (Cobble, 1987).

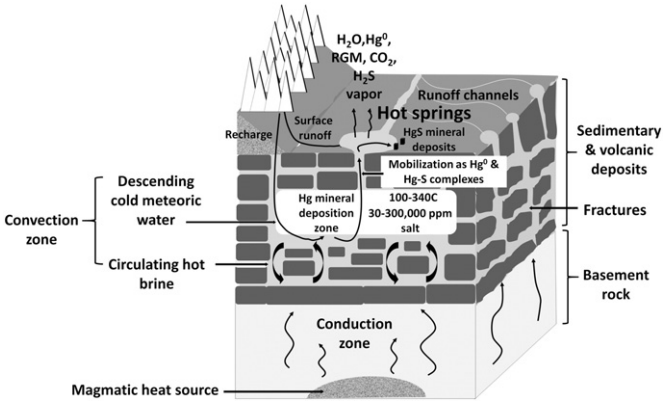


Fig. 1. Overview of mercury pathways and transformations in terrestrial geothermal systems as constructed from Sabadell and Axtmann, 1975; Varekamp and Buseck, 1984; Varekamp and Bruseck, 1986.

Biota inhabiting geothermal springs has had to adapt to the high concentrations of Hg in many of these systems. Hot springs microorganisms in particular have had to evolve strategies to deal with the toxic effects of high Hg concentrations under the various conditions that exist in these habitats. Molecular and phylogenetic analyses clearly show that the MerA protein, whose homodimer forms the active mercuric reductase enzyme which specifies Hg detoxification among many prokaryotes, first evolved in thermophilic microbes in geothermal environments (Barkay et al., 2010) prior to its spread to broadly distributed mesophilic microbes (Boyd and Barkay, 2012). The Mer detoxification system involves reduction of the inorganic Hg to the elemental volatile form, Hg(0), an activity found in several thermophilic microbes common in geothermal springs (Barkay et al., 2010). Thus, the activities of these microorganisms as well as temperature, pressure, and composition of solution affect the behavior (volatility, redox state, solubility) of Hg which impacts its fate and its influence on other life forms in the system. Here we synthesize information on the microbiology of hot springs of western NA, the relationships between the microbiology and the physicochemical properties, including Hg, and the MerA-mediated mechanism of Hg detoxification maintained by the microorganisms in these environments.

2. Microbiology of terrestrial geothermal systems of western North America

Hot springs of western NA have provided much information on the constraints of temperature and pH on microbial diversity. For example, temperature and pH have been shown to exert a strong influence on the diversity and types of microbial inhabitants in these systems (Miller et al., 2009; Purcell et al., 2007; Sharp et al., 2014). Correspondingly, the types of microorganisms inhabiting acidic hot springs differ greatly from those found in circumneutral thermal features (Reysenbach et al., 2005). Neutral-alkaline springs have consistently higher microbial diversity than acid springs, and acid spring communities are distinct from those in neutral and alkaline springs regardless of temperature (Sharp et al., 2014). Alkaline environments support greater algal diversity than acidic habitats, and diversity decreases regardless of pH when heavy metal concentrations are high (Amaral-Zettler, 2013). In general, the more extreme the environmental conditions, the lower the microbial diversity. However, the influence of Hg on the microbial communities in these systems is poorly understood.

2.1. Description of sites

Published information on prokaryotic taxa was collected from 271 geothermal sites across western NA with known pH, temperature and sulfide concentration, hereafter referred to as sites with both microbiological and physicochemical data. Of these sites, 44 had a pH > 8.0 (high pH sites), 141 sites had a pH ≥ 4.5 ≤ 8.0, (circumneutral sites) and 86

sites had a pH < 4.5 (acidic sites) (Table 1). Correspondingly, 38 of the 271 sites had a temperature < 55 °C (low temperature sites) 113 had a temperature between 55 and 73 °C (moderate temperature sites) and 120 had a temperature >73 °C (high temperature sites). The sites were further grouped according to sulfide concentration as sulfide is an important source of energy for a number of prokaryotic chemotrophic taxa in geothermal systems (Inskeep et al., 2013; Inskeep et al., 2010; Simbahan et al., 2005). Here, high sulfide sites are those with concentrations ≥3 μM while low sulfide sites are those with concentrations <3 μM. The 3 μM sulfide value reflects the highest detection limit encountered for a number of the sites included in the present survey (Inskeep et al., 2013; Jay, 2014; Klatt et al., 2013; Kozubal et al., 2012). Grouping of sites according to these pH and temperature ranges and sulfide concentration reflects the boundaries of key physiological processes for energy generation by microorganisms in geothermal systems (Boomer et al., 2009; Boyd et al., 2012; Brock, 1967; Castenolz et al., 1990; Kato et al., 2014; Klatt et al., 2013; Reysenbach et al., 2005; Ward et al., 1998).

2.1.1. Sites with mercury data

Mercury, which is common but not typically included in chemical surveys of hot springs, may also constrain microbial community diversity through its deleterious effects on cellular processes. Total Hg (THg) concentrations were obtained from the published literature for 56 or 21% of the 271 geothermal sites with microbiological and physicochemical data (SI Tables S1 and S2). The sites extend across five geomorphic provinces of western NA and include 27 acidic sites, 19 circumneutral sites and 10 high pH sites (Table 1). All but one of the acidic and circumneutral sites reside within Yellowstone National Park (YNP), WY, at or near the boundary between the Northern and Middle Rocky Mountain geomorphic provinces. The high pH sites are distributed between YNP and areas of California and Oregon within the Basin and Range geomorphic province. Thus, microbiological and physicochemical data that include Hg measurements are available for only a limited number of geothermal features across the region.

The 56 sites in which Hg has been measured exhibit a wide range of THg concentrations (SI Tables S1 and S2). Values range from 1.6 ng/L in circumneutral, moderate temperature, low sulfide, filtered water at a site within the Chocolate Pots thermal feature in YNP (Ball et al., 2008), to 24 μg/L in high pH, moderate temperature, high sulfide water of hot springs on Paoha Island in Mono Lake, CA (Mariner et al., 1977). Sites were further grouped according to THg concentration: low Hg sites with THg concentrations ≤100 ng/L and high Hg sites with THg concentrations >100 ng/L (Fig. 2). Mercury concentrations >100 ng/L are considered high for surface waters. Non-geothermal Hg-contaminated sites exhibiting Hg concentrations >100 ng/L have been shown to select for Hg-resistant microorganisms (Schaefer et al., 2004) as has Coso Spring, a low pH hot spring with mg/L concentrations of suspended cinnabar derived from a natural subsurface source (Simbahan et al., 2005). Since Hg availability and toxicity is influenced by pH, temperature and sulfide (Benoit et al., 2001; Benoit et al., 1999; Edgcomb et al., 2004; Farrell et al., 1990; Varekamp and Buseck, 1984), grouping of sites according to these environmental variables reflects Hg bioavailability.

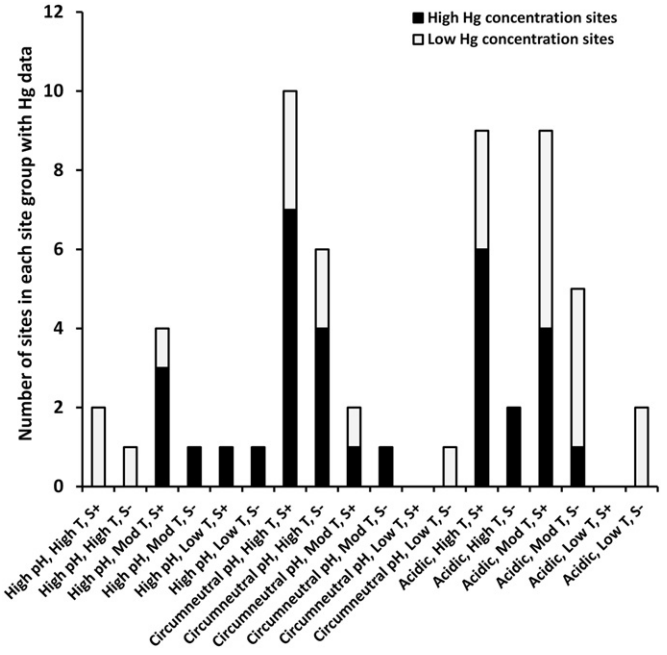


Fig. 2. Distribution of sites with Hg data across site groups. T refers to temperature, ModT refers to moderate temperature, S+ and S− refer to high and low sulfide concentration, respectively.

The site groups generally contain different numbers of sites with low and high THg concentration (Fig. 2). Several site groups contain only sites with high or low THg concentrations, while no Hg data is available for any of the sites in the circumneutral, low temperature, high sulfide group or any of the sites in the acidic, low temperature high sulfide group. The majority of high pH site groups have only one site in which Hg was measured (Fig. 2).

2.2. Distribution of prokaryotic taxa

A total of 245 prokaryotic taxa have been reported across all 271 geothermal sites with microbiological and physicochemical data (Table 1). Taxonomic assignments range from low resolution domain-level affiliations to high resolution genus-species identification, although genus is the highest resolution considered here (SI Figs. 1–3). Assignments are primarily based on 16S rRNA gene sequence analysis of isolates and environmental clone libraries and to a lesser extent on metagenomic analysis of samples collected from the sites.

The prokaryotic taxa reported across the 56 sites in which Hg was measured comprise 42% of the taxa distributed across all 271 sites (Table 1). Representation of taxa at sites with and without Hg data varies across the pH range. Of taxa reported across all 86 acidic sites 55% are present in one or more of the 27 sites in which Hg concentration was measured (Table 1). However, only 39% or 73 of the 187 taxa reported across all 141 circumneutral sites are present in the 19 sites in which Hg was measured, and only 21% or 19 of the 89 taxa reported

Table 1
Distribution of archaeal and bacterial taxa across pH site groups.

Site group	No. of sites	No. of archaeal taxa	No. of bacterial taxa	No. of prokaryotic taxa
All sites with microbiological & physicochemical data	271	83	162	245
All acidic sites with microbiological & physicochemical data	86	46	38	84
All circumneutral sites with microbiological & physicochemical data	141	55	132	187
All high pH sites with microbiological & physicochemical data	44	32	57	89
All sites with microbiological & physicochemical data including Hg	56	38	65	103
All acidic sites with microbiological & physicochemical data including Hg	27	27	19	46
All circumneutral sites with microbiological & physicochemical data including Hg	19	20	53	73
All high pH sites with microbiological & physicochemical data including Hg	10	3	16	19

across all 44 high pH sites are present in the 10 sites in which Hg was measured (Table 1). A plot of the number of taxa vs. number of Hg-containing sites investigated reveals a rarefaction curve showing that the number of taxa in Hg-containing sites will plateau far short of the 245 taxa detected across the 271 sites reported here (SI Fig 4). Nevertheless, it remains to be determined whether any of the taxa reported at one or more of the 271 sites that were not detected at any of the 56 sites in which Hg was measured reflected their sensitivity to Hg.

2.2.1. Distribution of Bacteria and Archaea

Bacterial taxa outnumber archaeal taxa across all 271 sites with microbiological and physicochemical data (Table 1). Specifically, bacterial taxa outnumber archaeal taxa across the 141 circumneutral and 44 high pH sites, whereas archaeal taxa outnumber bacterial taxa across the 86 acidic sites (Table 1). Correspondingly, richness of the bacterial community was higher than that of the archaeal community across the 10 high pH and 19 circumneutral sites with Hg data, while richness of the archaeal community was higher than that of the bacterial community across the 27 acidic sites in which Hg was measured (Table 1). Thus, the relative changes in bacterial and archaeal richness across the pH range of Hg-containing sites reflects the trend exhibited by the larger subset of geothermal features across the region. These data are consistent with previous reports of the dominance of Archaea in sulfidic, acidic systems and the dominance of Bacteria in low sulfide, circumneutral and high pH geothermal systems in western NA (Hugenholtz et al., 1998; Inskeep et al., 2013; Klatt et al., 2013; Meyer-Dombard et al., 2011). However, exceptions to these trends have been observed. DeLeon et al. (De Leon et al., 2013) recently reported higher than expected archaeal diversity at high pH sites in YNP, and Costa et al. (Costa et al., 2009) found comparable archaeal and bacterial richness in low sulfide circumneutral hot springs in the Basin and Range geomorphic province in Nevada.

Richness-based beta diversity, the effective number of distinct compositional units in a region, was determined for each site group. Beta diversity was standardized across site groups using the Sorensen index of dissimilarity to minimize the effect of differences in the number of sites in the groups on the diversity value (Chao et al., 2012). Index values for 12 of the 18 site groups range from 0.17 to 1.0 for archaeal communities and from 0.31 to 0.74 for bacterial communities (Table 2). The remaining site groups contain fewer than two sites or exhibit a beta diversity of 1, resulting in zero or negative index values. Table 2 also reveals patterns of dissimilarity across site groups. Dissimilarity is greater in archaeal than in bacterial communities in 8 of the 12 site groups. However, the difference is <23% (mean = 14 ± 6%) in 5 of these groups. Dissimilarity of archaeal communities exceeds that of bacterial communities by approximately a factor of two across all acidic and high pH, low temperature, low sulfide site groups, across all circumneutral, moderate temperature, low sulfide site groups and across all high temperature, high sulfide site groups. Dissimilarity of bacterial communities exceeds that of archaeal communities by more than a factor of two across all acidic, high temperature, low sulfide and across all moderate temperature low sulfide site groups.

Sulfide concentration has a greater effect on the dissimilarity of archaeal than bacterial communities across some temperature and pH ranges. When dissimilarity is greater in low than in high sulfide site groups with the same temperature and pH ranges, the difference is significantly greater ($p < 0.05$) for the archaeal communities (2.3 ± 0.30-fold, n = 3) than for the bacterial communities (1.3 ± 0.22-fold, n = 3) (Table 2). When dissimilarity is less in low than in high sulfide site groups with the same temperature and pH ranges, the difference for the archaeal communities (1.7 ± 0.22-fold, n = 2) is again greater than that for the bacterial communities (0.52-fold, n = 1). Thus, sulfide concentration appears to have a more pronounced effect on archaeal community dissimilarity than on bacterial community dissimilarity across these temperature and pH ranges.

Sorensen index values for communities associated with the different site groups were subjected to principal component analysis (PCA) using XLSTAT Version 2016.02.28540. The three pH ranges served as the variables and the different combinations of temperature and sulfide ranges served as the observations. Missing data for four observations were replaced using the mean or mode method. Dissimilarity in low and moderate temperature, low sulfide site groups and in high temperature high sulfide site groups account for most observation variance in the first dimension (F1) while dissimilarity in the moderate temperature high sulfide site group accounts for most of the observation variance in the second dimension (F2) (Fig. 3). The dissimilarity of archaeal communities in acidic and high pH sites accounts for most of the variance among variables, while dissimilarity of archaeal and bacterial communities associated with the low temperature, low sulfide sites accounts for most of the variance among observations in the first dimension (Fig. 3). The dissimilarity of archaeal and bacterial communities in circumneutral sites accounts for most of the variance among variables and that in moderate temperature, high sulfide sites accounts for most of the variance among observations in the second dimension (Fig. 3). The variance across these two dimensions accounts for 83% of the variance in community dissimilarity across site groups. The variance in dissimilarity of bacterial communities in high pH site groups accounts for much of the variance in high temperature, high sulfide site groups (Fig. 3). The dissimilarities of archaeal communities in acidic and high pH site groups are positively correlated (Pearson correlation = 0.89), as are the dissimilarities of archaeal communities in circumneutral site groups and bacterial communities in acidic site groups (0.86). In summary, the results show that pH, temperature and sulfide concentration collectively exert a large impact on beta diversity of archaeal and bacterial communities in these geothermal systems. The beta diversity trends exhibited by archaeal communities often differ from those of bacterial communities across the same range of pH, temperatures and sulfide concentrations in geothermal systems. It remains to be determined whether these trends are maintained as more microbial community

Table 2
Sorensen index of dissimilarity for prokaryotic communities in different site groups.

Site group	Archaeal communities			Bacterial communities		
	Acidic	Circumneutral	High pH	Acidic	Circumneutral	High pH
highT[a], highS[a]	0.17 (16)[b]	0.66 (15)	0.75 (3)	0.64 (6)	0.38 (23)	0.67 (3)
highT, lowS	0.42 (6)	0.43 (23)	ND[c] (1)	ND (1)	0.37 (26)	0.48 (5)
modT, highS	0.49 (16)	0.40 (3)	ND (1)	0.46 (11)	0.31 (10)	ND (1)
modT, lowS	0.26 (15)	0.79 (8)	0.61 (9)	0.69 (9)	0.45 (9)	0.52 (11)
lowT, highS	0.40 (2)	ND (1)	ND (0)	0.60 (2)	0.67 (5)	ND (0)
lowT, lowS	1.0 (3)	ND (0)	1.0 (2)	0.56 (6)	0.74 (6)	0.47 (6)

[a] T and S refer to temperature and sulfide concentration, respectively.
[b] Number of sites is in parenthesis.
[c] No data.

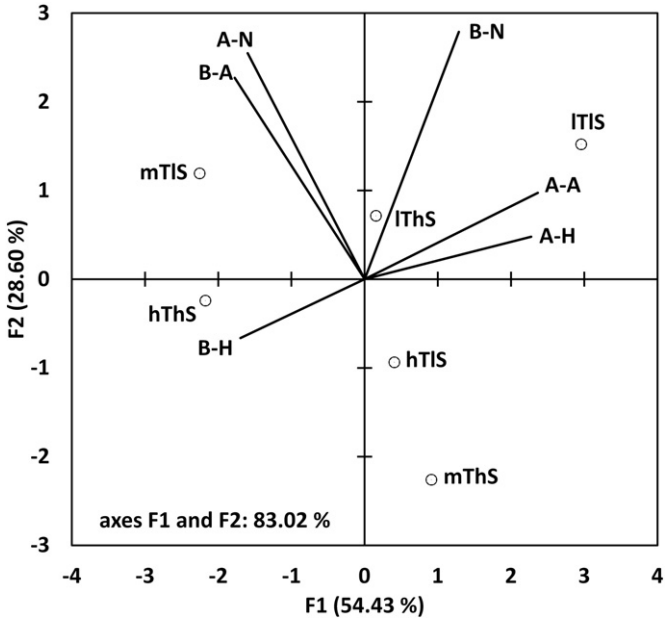


Fig. 3. Principal component analysis constructed with beta diversity dissimilarity transformed into Sorensen index values. Principal component 1, F1 and principal component 2, F2, plotted against each other, with a total of 83.02% of variation explained. A-A = archaeal-acidic pH, A-N = archaeal-circumneutral pH, A-H = archaeal-high pH, B-A = bacterial-acidic pH, B-N = bacterial-circumneutral pH, B-H = bacterial-high pH, hThS = high temperature, high sulfide concentration, hTlS = high temperature, low sulfide concentration, mThS = moderate temperature, high sulfide concentration, mTlS = moderate temperature, low sulfide concentration, lThS = low temperature, high sulfide concentration, lTlS = low temperature, low sulfide concentration.

and physicochemical data are obtained for sites that fall within the underrepresented site groups.

2.2.2. Prokaryotic taxa at acidic sites containing Hg

A number of taxa appear to be common inhabitants of Hg-containing geothermal systems. Of the 46 taxa reported across all 27 acidic sites in which Hg was measured, 7 Archaea and 4 Bacteria taxa were detected at three or more sites (SI Table 3). That some taxa are reported at more sites than other taxa to some extent reflects the inclusion of data from distribution studies of specifically targeted taxa (Clingenpeel et al., 2013; Kozubal et al., 2008; Miller-Coleman et al., 2012; Papke et al., 2003; Reysenbach et al., 2005; Ross et al., 2012; Takacs-Vesbach et al., 2013; Takacs-Vesbach et al., 2008; Tin et al., 2011; Whitaker et al., 2003).

The genus *Hydrogenobaculum* (domain Bacteria, phylum *Aquificae*) is not only the most frequently reported taxon across all 86 acidic sites (present at 29 or 34% of the sites) with microbiological and physicochemical data (SI Fig. 1a–f), but is also the most frequently reported taxon across the 27 acidic sites (detected at 14 or 52% of the sites) in which Hg was measured (SI Table 3). The majority of the sites (9 of 14) in which this genus has been reported are high Hg sites. The majority (11) of these 14 sites also have a high or moderate temperature and high sulfide concentration; conditions that favor Hg volatilization and precipitation which reduce Hg bioavailability (Benoit et al., 2001; Benoit et al., 1999; Varekamp and Buseck, 1983; Varekamp and Buseck, 1984). The high frequency of occurrence of *Hydrogenobaculum* at sites with these characteristics is consistent with its widespread distribution across acidic, sulfur-containing geothermal habitats (Burgess et al., 2012; Donahoe-Christiansen et al., 2004; Mathur et al., 2007; Reysenbach et al., 2005).

Among Archaea, the genus *Metallosphaera* (phylum *Crenarchaeota*) is a common inhabitant of acidic geothermal sites, particularly those containing iron (Kozubal et al., 2008). The genus has been reported in 39 (45%) of the 86 acidic sites with microbiological data (SI Fig. 1a–f) and in 5 or 19% of the 27 sites in which Hg was also measured, the majority of which (4 of 5) are high Hg sites (SI Table 3). Other taxa detected at three or more acidic, Hg-containing sites include the crenarchaeotes *Acidianus*, *Thermocladium* and *Thermoproteus*, the actinobacteria *Acidimicrobium* and the firmicutes *Alicyclobacillus* and *Sulfobacillus*. To date, the sites at which *Acidimicrobium* has been reported are all low Hg sites, whereas at least one of the sites in which the other taxa have been reported is a high Hg site. Other taxa detected at more than one low Hg site include Group I.1-e *Thaumarchaeota* and the proteobacteria *Desulfurella* (SI Fig. 1a–d, f). To date, the sites in which Hg was measured that contain Group I.1-e *Thaumarchaeota* and *Desulfurella* are all low Hg sites (SI Table 3). Additional studies are needed to determine whether some prokaryotic taxa are unable to tolerate the high THg concentrations that occur at some acidic geothermal sites. However, the limited data to date suggest that a number of archaeal and bacterial taxa tolerate the high THg concentrations at many acidic geothermal sites.

2.2.3. Prokaryotic taxa at circumneutral sites containing Hg

There are fewer total prokaryotic taxa but more bacterial taxa reported at high frequency (3 or more sites) across all 19 circumneutral sites in which Hg was measured than are reported across the 27 acidic sites in which Hg was measured. Of the 73 taxa reported across the 19 circumneutral sites, 1 Archaea and 5 Bacteria are reported at high frequency (SI Table 4). The archaeon *Vulcanisaeta* is the only genus reported at high frequency across acidic and circumneutral sites in which Hg was measured, all but one of which are high Hg sites. Similarly, the majority of circumneutral sites supporting the frequently encountered chemotrophic Bacteria *Thermus*, *Hydrogenothermus* and *Thermocrinus* are high Hg sites. By contrast, the majority of circumneutral sites supporting the frequently reported phototrophic Bacteria *Chloroflexus* and *Synechococcus* are low Hg sites. Additional studies are needed to determine whether phototrophs are more sensitive to Hg than chemotrophs in these circumneutral geothermal systems.

2.2.4. Prokaryotic taxa at high pH sites containing Hg

Of the 19 prokaryotic taxa reported across all 10 high pH sites in which Hg was measured, only the cyanobacterium *Synechococcus* was detected at high frequency (SI Table 5). *Synechococcus* ecotypes have been shown to inhabit microbial mats at a number of circumneutral geothermal sites in western NA, particularly those with low to moderate temperatures (Papke et al., 2003). The seven high pH sites in which *Synechococcus* was detected are distributed across all temperature and sulfide groups except the low temperature, high sulfide group (SI Table 5). The data suggest that *Synechococcus* tolerates low THg concentrations in the presence of high and low sulfide concentrations at high temperatures, high Hg concentrations in the presence of high and low sulfide concentrations at moderate temperatures, and high Hg concentrations in the presence of high sulfide concentrations at low temperatures (SI Table 5). Thus, *Synechococcus* may be less tolerant of high Hg concentrations at high temperatures than at moderate and low temperatures.

Of the high pH sites in which Hg was measured, those with low Hg contain the majority of taxa (16 of 19) (SI Table 5). Only Group 1 *Crenarchaeota*, the γ-proteobacteria *Ectothiorhodospira* and the cyanobacterium *Oscillatoria* were reported at high pH sites with high THg concentrations (SI Table 5). The results suggest that across the pH range of geothermal systems in NA, archaeal and bacterial richness appears most constrained by Hg concentration under high pH conditions.

3. Potential for Hg reduction and detoxification in terrestrial hot springs in western North America

Mercury is a widespread and persistent toxic element that when converted to its methylated form, the potent neurotoxic substance monomethylmercury (MeHg), is bioaccumulated and biomagnified

through organisms in food chains. At the top of the food chain, therefore, organisms may contain extremely high MeHg concentrations leading to neurological damage and sometimes death (Driscoll et al., 2013). Microorganisms play an important role in modulating MeHg formation in the environment by methylating inorganic Hg to MeHg, degradation MeHg, and carrying out redox transformations that control the availability of inorganic Hg to methylating microbes (Barkay et al., 2003).

While human activities have added Hg to many environments, particularly since the advance of the industrial revolution, [reviewed by (Driscoll et al., 2013)], natural processes are responsible for Hg enrichment in geothermal environments above the element's average abundance in the earth crust (see above). This fact raises the interesting hypothesis that thermophilic microbes evolved in the presence of Hg in geothermal environments (Barkay et al., 2010; Vetriani et al., 2005) creating a genetic potential that, when challenged by increased anthropogenic Hg inputs elsewhere, could spread to diverse microbes in more recently Hg-impacted environments (Boyd and Barkay, 2012). While this hypothesis was tested by analyzing the composition of mercury resistance operons (*mer* operons) in sequenced microbial genomes (Boyd and Barkay, 2012), here we examine this possibility by considering abundance and distribution of MerA, the mercuric reductase enzyme, in metagenomes from geothermal environments. Mercury methylation is not considered in this paper as there is little information currently available on methylation by microorganisms in hot springs and on the effect of MeHg on life in geothermal environments.

3.1. mer operon-mediated Hg resistance

Mercury is the most toxic metal to microorganisms inhibiting growth in common laboratory media when a concentration of a few μM is exceeded (Nies, 1999). The major mechanism of this toxicity is the consequence of the very high affinity of Hg to thiol moieties leading to the distortion of protein structure and loss of activity. To survive this high toxicity, microbes may employ an elaborate resistance system, encoded by the *mer* operon, whereby exposed cells actively transport inorganic Hg into the cell where it is reduced to the elemental form, Hg^0, by MerA. Because Hg^0 is lipophilic and highly volatile (Henry's constant 0.3 dimensionless) and has low aqueous solubility (6 μg per 100 mL of water at 25 °C), it readily diffuses out of the cell and partitioned into the gaseous phase and away from the immediate environment of the resistant microbe (Barkay et al., 2003).

The *mer* operon, initially characterized among common mesophilic bacteria (Smith, 1967), was more recently described among several thermophilic bacteria (Freedman et al., 2012; Wang et al., 2009) and Archaea (Artz et al., 2015; Schelert et al., 2004) from deep-sea hydrothermal vents and marine and terrestrial hot springs. We have assessed the role of the *mer* operon in Hg detoxification in terrestrial hot springs by examining the abundance of MerA homologs, specifying the central function of the *mer* operon (Barkay et al., 2003) in the genomes of microbes common in hot springs (Section 3.1.1 and SI Table 6) and in metagenomes of microbial mats (Section 3.1.2 and SI Table 6).

3.1.1. Occurrence of MerA in microbial taxa found in geothermal systems of western North America

The potential for MerA to increase microbial fitness in hot springs was examined by looking for MerA in the genomes of microbes that have been reported in geothermal systems by homology searches of their translated complete microbial genomes. Among 245 bacterial and archaeal taxa detected across hot springs in western NA (SI Figs. 1–3), at least 9 archaeal and 29 bacterial taxa had at least one representative where MerA was present (SI Table 6). The remaining included taxa for which complete genome sequences are not yet available or homology searches failed to detect MerA. The distribution of MerA-carrying taxa in the different classes of springs shows interesting trends:

1. At low pH springs archaeal and bacterial taxa have similar representation of MerA while bacterial MerA-carrying taxa dominate at higher pH springs (Fig. 4). Moreover, archaeal taxa with MerA homologs are particularly rare in cooler springs, i.e., <55 °C. This distribution may simply reflect taxa abundance as metagenomic analysis indicates that at pH <4.5 microbial surface-associated communities consist mostly of Archaea and the bacterial order *Aquificales*, whereas Bacteria are common in less acidic springs (Inskeep et al., 2013).
2. Consistent with prior observations on the intolerance of *Cyanobacteria* to low pH (Brock, 1973), phototrophic taxa, both anoxygenic and oxygenic, are not present in springs with pH <4.5 (SI Table 6). While homologs of the broadly-distributed Tn*501*-like MerA (Barkay et al., 2003) and the newly discovered *Synechocystis*-like MerA (Marteyn et al., 2013) were detected among cyanobacterial taxa, low levels of homology (e value of ~50 for the entire protein sequence) were noted. For example, the presence of only 9 of 16 conserved amino acid residues in the redox active site of MerA and absence of amino acid residues essential for MerA activity (Barkay et al., 2010; Boyd and Barkay, 2012) question the functionality of Tn*501*-like MerA among *Chloroflexi* and the *Cyanobacteria*. The environmental significance of the *Synechocystis* MerA, strongly suggested by the occurrence of taxa with this locus across a broad range of ecological niches (SI Table 6), is presently unknown.

3.1.2. Relationships between occurrence of MerA in microbial communities and geothermal spring pH

The occurrence of MerA homologs in the metagenomes of microbial communities from hot springs in the northwestern U.S. was determined by searching the Joint Genome Institute (JGI) translated metagenomes databases (https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=FindGenesBlast&page=geneSearchBlast) using the protein sequence of the prototypic Tn*501* MerA (accession number CAA77323; Fox, 1982 #136}. At the time of this analysis, April 2015, there were 64 such metagenomes in the database (SI Table 7) with a total of 23,811,152 genes, 165 of which were homologs of MerA giving an overall frequency of 1 homolog/1.4×10^5 genes. It will be interesting to see if

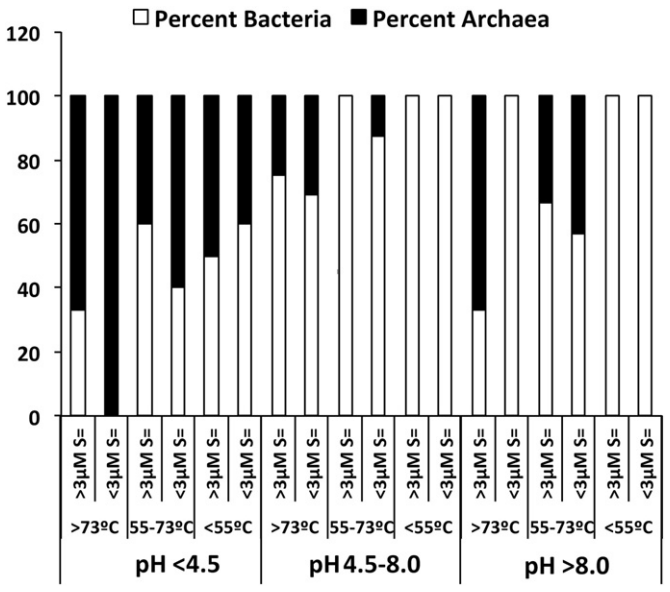


Fig. 4. The distribution of bacterial and archaeal MerA-carrying taxa in hot springs in NA. MerA homologs were detected by a blastp search using https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=FindGenesBlast&page=geneSearchBlast. MerA of Tn*501* was used as a query and the search was constrained to *e* values of $<10^{-50}$.

MerA frequency correlates with the concentration of Hg as more Hg data become available.

3.1.2.1. pH-dependent distribution of MerA in metagenomes.. A clear inverse correlation is observed between spring pH and the frequency of MerA homologs in the community metagenome gene pool (Fig. 5A). Springs at pH <4.5 had an average of 102 MerA homologs/10^6 genes, those at pH of 4.5–8.0, 14 MerA/10^6 genes and the single spring at a pH >8.0 where MerA was detected had 12 MerA/10^6 genes. Note that this analysis includes only metagenomes where MerA was detected and those with $>10^4$ total gene counts. In addition, at pH < 4.5 MerA homologs are present in most metagenomes (17 of 21), at lower frequency (8 of 22) at circumneutral pH, and in only 1 of 5 metagenomes from high pH sites (Fig. 5B).

We performed PCA analysis to further examine the relationships between MerA frequency and spring pH and temperature. There was not enough springs with known sulfide and Hg concentrations to include these variables in the analysis. A PCA biplot of this frequency as a function of spring pH and temperature clearly shows the opposing directions of MerA distribution and spring pH (Fig. 5C). In fact, five of the seven highest frequencies of MerA, 124 to 154 $\times$ 10^{-6} MerA/gene) are in springs with pH < 4.5 whereas the lowest MerA frequencies (7 to 26 $\times$ 10^{-6}), are associated with six of eight springs with pH of 5.7 to 7.6. Temperature has a slight positive effect on MerA frequency over the temperature range of 53 °C to 90 °C, which spans all sites for which metagenome sequences were available (SI Table 7). Thus, acidic springs are more likely to have populations containing MerA homologs. This relationship may be related to the higher concentration of dissolved total Hg in the water of low, as compared to higher, pH springs (D.K. Nordstrom, personal communication) suggesting selection of taxa that possess MerA in the springs. This abundance of MerA in metagenomes from acidic springs is consistent with the frequent presence of MerA in the genomes of acidophilic Archaea (SI Table 6) (Barkay et al., 2010; Boyd and Barkay, 2012), and with the definition of low pH springs as archaeal systems (Inskeep et al., 2013). Similar analyses relating spring temperature to presence and abundance of MerA showed no significant trends or patterns.

3.1.2.2. MerA type distribution. All MerA homologs were identified by their similarity to known MerA sequences using tblastn searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). Most homologs are most closely related to archaeal sequences (Fig. 6) and they are related to four order-specific groups, two within the *Crenarchaeota* (*Sulfolobales* and *Thermoproteales*), one within the *Euryarchaeota* (*Thermoplasmatales*) and one within the *Thaumararchaeota* (*Nitrososphaerales*). Bacterial homologs are very rare and the majority are found in a single very large metagenome from Obsidian Pool where about half of all homologs are bacterial.

When the class-level distribution is considered relative to pH of the spring water, some clear patterns emerge (Fig. 7). *Sulfolobales*-type MerA sequences were mostly present in low pH springs while the *Nitrososphaerales*-type sequences were not present at any spring with a pH < 3.0. Interestingly, Beam et al. (Beam et al., 2014) reported dominance of *Thaumarchaeota*-related sequences in the metagenomes of low pH Yellowstone springs (pH 2.9–3.1). This apparent discrepancy may simply be due to the fact that *Nitrososphaerales*-like MerA were identified here by their closest similarity to MerA of *Candidatus Nitrososphaera gargensis* Ga9.2 (SI Table 6), a strain that was isolated from a hot spring with slightly alkaline pH (Lebedeva et al., 2005). Thus, as was observed with pure cultures (see section 4.1.1), MerA distribution followed the distribution of taxa most notably with regard to sequences affiliated with the *Sulfolobales* and their preference in low pH springs (Inskeep et al., 2013).

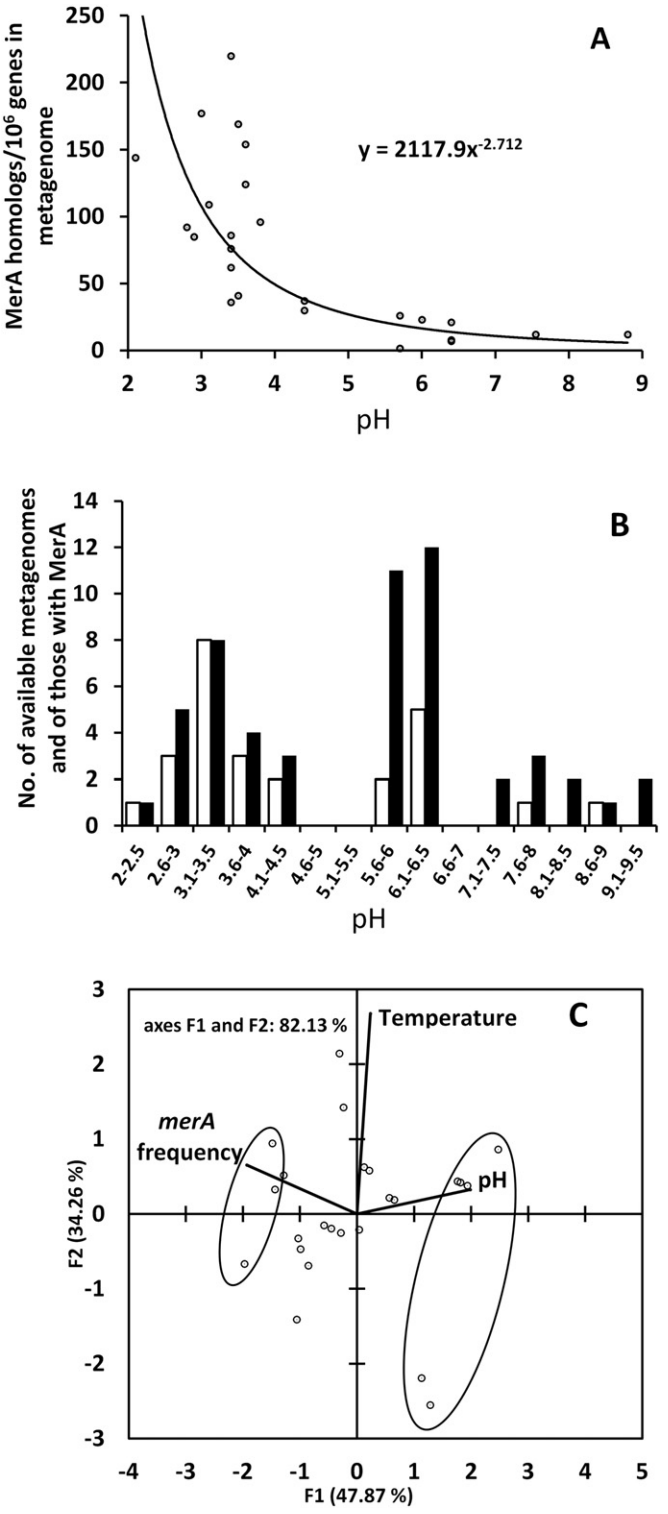


Fig. 5. Relationships between MerA distribution and spring physicochemical parameters. (A) frequency of MerA in metagenomes in which MerA was detected as a function of site pH, (B) the number of metagenomes in which MerA was detected (unfilled bars) and the number of metagenomes (filled bars) available from sites with a pH within the indicated range, (C) principal component analysis constructed with site MerA frequencies, temperature and pH. Principal component 1, F1 and principal component 2, F2, plotted against each other, with a total of 83.02% of variation explained.

3.1.2.3. Relationship of MerA occurrence to taxa detected at acidic sites. Previous studies suggest that some taxa of the *Aquificae*, including *Hydrogenobaculum* possess early forms of the *mer* operon (Barkay et al., 2010; Freedman et al., 2012; Romano et al., 2013). However, a

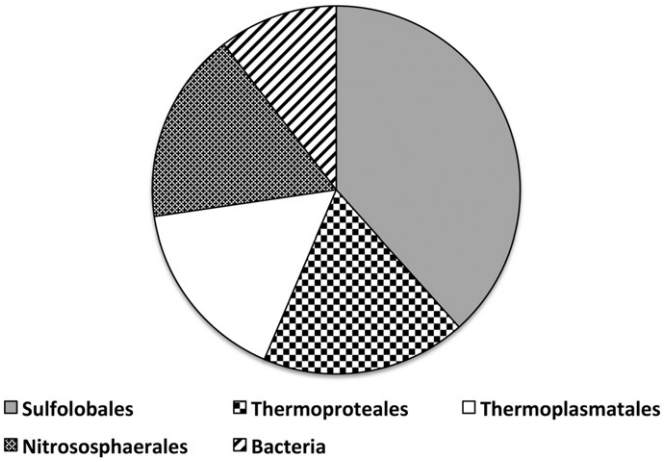


Fig. 6. Order-specific affiliation of MerA homologs from hot springs microbial communities. Homologs were sorted by the taxonomic order of their most closely related MerA sequence.

MerA homolog most closely affiliated with *Hydrogenobaculum* has yet to be reported at a site in which both the taxon and Hg are found. This taxon's close association with elemental sulfur in acidic geothermal systems may offer a different means of protection, possibly by occupying a niche where Hg bioavailability is reduced, precluding the need for MerA under these conditions.

Metagenomic studies have also detected gene sequences most closely affiliated with MerA of the crenarchaeote *Metallosphaera* at four of the acidic sites in which the genus was detected and Hg was measured (SI Fig. 1a, c and d). Given its frequency and abundance at Hg-containing sites, *Metallosphaera* may play a significant role in Hg reduction in its habitat. MerA most closely affiliated with other crenarchaeotes (*Sulfolobus*, *Caldiverga* and *Vulcanisaeta*) have been reported at one or more of the same sites in which these genera were detected (SI Fig. 1a and d, SI Table 8). All three genera have been reported at low and high Hg sites (SI Table 3). Thus, lineages of the *Crenarchaeota* may be important mediators of Hg reduction in acidic geothermal systems.

3.1.2.4. Relationship of MerA occurrence to taxa detected at circumneutral sites. MerA have also been detected for less frequently encountered taxa at circumneutral sites with Hg data. MerA most closely affiliated with the crenarchaeote *Pyrobaculum* and the *Aquificae Hydrogenobacter* have been detected at high temperature, high sulfide, high-Hg sites in which these genera have been reported (SI Fig. 2a-1 and a-2, SI Table 8). Thus, MerA homologs are distributed across bacterial and archaeal taxa inhabiting circumneutral as well as acidic high temperature, high sulfide sites.

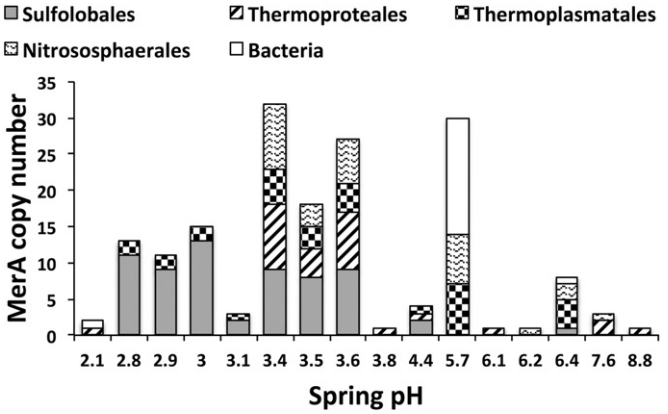


Fig. 7. Distribution of MerA homologs as a function of spring pH.

3.1.2.5. Relationship of MerA occurrence to taxa detected at high pH sites. MerA have been detected in two high pH geothermal systems in YNP: one affiliated with *Pyrobaculum* in Conch Spring (SI Fig. 3a, SI Table 8) and one affiliated with *Rhodothermus* at low Hg Bijah Spring (Wang et al., 2011). No Hg data is yet available for Conch Spring and no taxon closely related to *Rhodothermus* has yet been detected in Bijah Spring. Thus, the significance of MerA in high pH geothermal systems remains to be determined. To date, MerA has been reported for only a small fraction of the taxa detected at Hg-containing geothermal systems. The majority of taxa inhabiting these systems may therefore utilize MerA that have evaded detection by protein homology studies or because they utilize an alternative Hg resistance system.

3.2. Relating MerA distribution and frequency in hot spring microbial communities to its global distribution and potential utilization in Hg bioremediation

Of the mechanisms known to confer Hg resistance to microorganisms (Barkay et al., 2003; Hidalgo et al., 2010; Latinwo et al., 1998) the MerA-mediated system is the most successful Hg detoxification known to date. Phylogenetic reconstructions strongly support an origin and early evolution of MerA, the central function of the *mer* operons, in geothermal environments (Barkay et al., 2010) and subsequent developments by localization on plasmids, addition of transport functions, and increasing sophistication of regulatory circuits that tightly control *mer* operon expression (Boyd and Barkay, 2012). The result is a highly efficient Hg detoxification system that is broadly distributed among obligate and facultative aerobic microbes in all environments and provides the important ecosystem service of converting Hg(II) to Hg(0) in those environments where Hg(II) may exist at toxic levels (Barkay et al., 2010). Furthermore, *mer* operon activities are used to treat Hg-laden wastes (Wagner-Döbler et al., 2000b), for the construction of bioreporters to detect bioavailable Hg(II) (Chiasson-Gould et al., 2014) and to manufacture Hg-specific biosorbents (Kostal et al., 2003).

By investigating patterns of MerA distribution among microbial taxa in geothermal environments where this protein originated, it should be possible to identify conditions that constrain MerA success as a detoxification system. For example, in Sections 3.1.2.2 to 3.1.2.5 above and in Fig. 7, MerA distribution followed taxa distribution suggesting that taxon fitness determines MerA distribution, a pattern that extends to global distribution of MerA (Barkay et al., 2010; Boyd and Barkay, 2012). In man-impacted environments one expects, and indeed observes (Wagner-Döbler et al., 2000a) that it is the indigenous members of the community, rather than strains genetically engineered to optimize *mer* operon activities (Horn et al., 1994) that dominate Hg removal. The strong relationship between MerA frequency and pH revealed above (Section 3.1.2.1 and Fig. 7) supports other studies which have documented the influence of pH on Hg bioavailability (Kelly et al., 2003). This knowledge should provide valuable insight on the conditions under which the *mer* operon activities will be most successful as a bioremediation strategy.

4. Summary

Microbiological and physicochemical data that include Hg measurements are available for a limited number of geothermal features across western NA. Those features in which Hg has been reported, the majority of which contain sulfide and have high or moderate temperature and circumneutral or acidic pH, exhibit a wide range of Hg concentrations. Acidic, Hg-containing sites have the greatest portion of taxa reported across acidic sites for which microbiological data is available while high pH, Hg-containing sites have the smallest portion of taxa reported across the corresponding high pH sites. It remains to be determined whether microbial communities at high pH sites contain a higher percentage of Hg-sensitive taxa than those at acidic sites. However, bacterial and archaeal genera known to carry MerA, the enzyme that

specifies resistance to Hg, have been isolated from many hot springs with archaeal taxa dominating in low pH and bacterial taxa dominating in circumneutral and high pH springs. Interestingly, phototrophic taxa with MerA homologs are only detected in circumneutral and high pH sites. Thus, a potential for *mer*-operon mediated Hg detoxification exists in geothermal springs and its distribution depends on taxon distribution as constrained by physical and chemical factors.

Richness of bacterial communities is greater than that of archaeal communities across Hg-containing circumneutral and high pH sites while the opposite is observed across Hg-containing acidic sites, a pattern that extends across the broader geothermal landscape irrespective of the presence of Hg. Richness-based beta diversity of bacterial and archaeal communities varies across the pH, temperature and sulfide concentration range of geothermal systems in NA. Sulfide concentration appears to have a greater effect on archaeal than on bacterial community dissimilarity across some temperature and pH ranges. The beta diversity trends exhibited by archaeal communities often differ from those of bacterial communities across the same range of pH, temperatures and sulfide concentrations in these systems.

The taxa most commonly encountered within the Hg-containing acidic, circumneutral and high pH site groups generally differ from one another. The exceptions are *Vulcanisaeta* which is common across both acidic and circumneutral site groups and *Synechococcus* which is common across both circumneutral and high pH site groups.

Homologs of MerA are present in microbial community metagenomes from 16 of 48 springs for which metagenomes were available. Low pH springs emerge as an environment favoring MerA as (i) most metagenomes from such springs contain MerA, (ii) MerA is more abundant in acidic than circumneutral and high pH springs, and (iii) PCA analysis clearly shows an inverse relationship between spring pH and MerA frequency. These relationships are likely related to higher concentrations of dissolved Hg, and thus Hg bioavailability, in low pH springs.

Most MerA homologs detected in metagenomes from hot springs in the western U.S. are most similar to those of four archaeal orders common in geothermal springs, including *Sulfolobales* which dominate acidic springs and *Nitrososphaerales* which are restricted to springs with pH >3.0. The MerA homologs most similar to those of Bacteria come from one large metagenome from a circumneutral spring. These patterns are only partially explained by known distribution patterns of microbial taxa, highlighting our limited understanding of factors that constrain taxa distribution and Hg bioavailability in geothermal ecosystems.

5. Challenges

An understanding of the interactions between Hg and microorganisms in geothermal systems, while assisted in recent decades by improvements in molecular methods, still faces major challenges. Some challenges are specific to geothermal systems while others apply to many environments where Hg and microorganisms coexist.

1. Microbial communities in geothermal systems often exist in sediments or as substratum-associated biofilms or mats. Strong chemical gradients develop within these sessile communities due to mass transport limitations. Thus, the chemical conditions (i.e., pH, sulfide and Hg concentration) to which these communities are exposed typically differ from what is measured in the overlying bulk aqueous phase.
2. We do not yet know the spatial boundaries of a functional community residing in the chemical gradients of these systems. Consequently, we do not know the concentrations of a chemical to which the community is exposed. Even if the area or volume of a functional community can be defined, the size of a sample may not be large enough for chemical analysis.
3. The communities are not only influenced by the chemistry of the overlying bulk aqueous phase but also by the chemistry and mineralogy of the solid phase with which they are associated (Ramsing et al., 2000; Reardon et al., 2004; Ruff-Roberts et al., 1994). Some of the same aqueous phase elements that influence community composition may also be present in the solid phase (i.e., sulfide and Hg in the form of cinnabar or metacinnabar). Furthermore, unknown heterogeneities on the surface of the solid phase may also influence community distribution.
4. The temperature and chemistry of many geothermal features vary over time. The composition of the microbial community is likely to change in response to these environmental variables. Sampling for microbiological and chemical characterization, including Hg, needs to be carried out simultaneously across different time scales to determine how variations in Hg concentration affect community composition.
5. Geothermal systems often contain both soluble chemicals and particulate mineral phases that react strongly with Hg. The interactions of Hg with dissolved organic matter and sulfides are currently considered to drive Hg bioavailability in both soluble and mineral phases in sedimentary environments (Hsu-Kim et al., 2013). While, it is likely that these interactions also influence Hg bioavailability in geothermal microbial communities, the critical combined effects of pH and temperature on taxa and MerA distributions clearly highlight the need to integrate factors unique to geothermal systems into existing paradigms of Hg bioavailability. Thus, the study of such systems, provides unique opportunities to expand our understanding of Hg biogeochemistry in environments, where microbial interactions with this toxic metal has been evolving for billions of years.

6. Future directions for research

Microorganisms appear to have been exposed to Hg early in their evolution during colonization of geothermal habitats (Barkay et al., 2010). The interactions that have evolved between Hg and microorganisms in these systems over time likely affect those which have developed more recently in environments impacted by anthropogenic inputs of Hg. Perhaps the most important thing learned from the current synthesis is the need for a systematic coordinated interdisciplinary research approach to microbiological and geochemical sampling and analysis, including Hg and its various forms in geothermal systems. This approach should fill many of the gaps in our understanding of microbe-Hg interactions and provide a clearer picture of biogeochemical cycling of Hg in these systems.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.06.080.

Acknowledgments

Funding to T.B. was provided by NSF's Geobiology and Low-Temperature Geochemistry program, EAR-0952291 and by a Hatch/McIntyre-Stennis grant through the New Jersey Agricultural Experiment Station.

References

Amaral-Zettler, L.A., 2013. Eukaryotic diversity at pH extremes. Front. Microbiol. 3. http://dx.doi.org/10.3389/fmicb.2012.00441.

Artz, J.H., White, S.N., Zadvornyy, O.A., Fugate, C.J., Hicks, D., Gauss, G.H., et al., 2015. Biochemical and structural properties of a thermostable mercuric ion reductase from *Metallosphaera sedula*. Front. Bioeng. Biotechnol. 3, 97.

Ball, J.W., McCleskey, R.B., Nordstrom, D.K., Holloway, J.M., 2008. Water-chemistry data for selected springs, geysers, and streams in Yellowstone National Park, Wyoming, 2003–2005. Open-file Report 2006-1339. U.S. Geological Survey, Washington, D.C., p. 137.

Barkay, T., Miller, S.M., Summers, A.O., 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27, 355–384.

Barkay, T., Kritee, K., Boyd, E., Geesey, G., 2010. A thermophilic bacterial origin and subsequent constraints by redox, light and salinity on the evolution of the microbial mercuric reductase. Environ. Microbiol. 12, 2904–2917.

Beam, J.P., Jay, Z.J., Kozubal, M.A., Inskeep, W.P., 2014. Niche specialization of novel Thaumarchaeota to oxic and hypozic acid geothermal springs of Yellowstone National Park. ISME J. 8, 938–951.

Becker, G.F., 1888. Geology of the quicksilver deposits of the Pacific slope, with an atlas. Monograph Report No. 13. U.S. Government Printing Office, Washington, D.C.

Benoit, J.M., Gilmour, C.C., Mason, R.P., Heyes, A., 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. Environ. Sci. Technol. 33, 951–957.

Benoit, J.M., Gilmour, C.C., Mason, R.P., 2001. The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of *Desulfobulbus propionicus* (1pr3). Environ. Sci. Technol. 35, 127–132.

Berry, G.W., Grim, P.J., Ikelman, J.A., 1980. Thermal springs list for the United States: key to geophysical records documentation no. 12. In: Commerce, U.S.D. (Ed.), National Oceanic and Atmospheric Administration. National Geophysical and Solar-Terrestrial Data Center, Boulder, CO.

Boomer, S.M., Noll, K.L., Geesey, G.G., Dutton, B.E., 2009. Formation of multilayered photosynthetic biofilms in an alkaline thermal spring in Yellowstone National Park, Wyoming. Appl. Environ. Microbiol. 75, 2464–2475.

Boyd, E.S., Barkay, T., 2012. The mercury resistance operon: from an origin in a geothermal environment to an efficient detoxification machine. Front. Microbiol. 3. http://dx.doi.org/10.3389/fmicb.2012.00349.

Boyd, E.S., Fecteau, K.M., Havig, J.R., Shock, E.L., Peters, J.W., 2012. Modeling the habitat range of phototrophs in Yellowstone National Park: toward the development of a comprehensive fitness landscape. Front. Microbiol. 3. http://dx.doi.org/10.3389/fmicb.2012.00221.

Brock, T.D., 1967. Relationship between primary productivity and standing crop along a hot spring thermal gradient. Ecology 48, 566–571.

Brock, T.D., 1973. Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. Science 179, 480–483.

Burgess, E.A., Unrine, J.M., Mills, G.L., Romanek, C.S., Wiegel, J., 2012. Comparative geochemical and microbiological characterization of two thermal pools in the Uzon Caldera, Kamchatka, Russia. Microb. Ecol. 63, 471–489.

Castenolz, R.W., Bauld, J., Jorgensen, B.B., 1990. Anoxic microbial mats of hot springs: thermophilic *Chlorobium* sp. FEMS Microbiol. Ecol. 74, 325–336.

Chao, A., Chiu, C.-H., Hsieh, T.C., 2012. Proposing a resolution to debates on diversity partitioning. Ecology 39, 2037–2051.

Chiasson-Gould, S.A., Blais, J.M., Poulain, A.J., 2014. Dissolved organic matter kinetically controls mercury bioavailability to bacteria. Environ. Sci. Technol. 48, 3153–3161.

Clingenpeel, S., Kan, J., Macur, R.E., Woyke, T., Lovalvo, D., Varley, J., et al., 2013. Yellowstone lake nanoarchaeota. Front. Microbiol. 4. http://dx.doi.org/10.3389/fmicb.2013.00274.

Cobble, J.W., 1987. A theory on mercury in geothermal fluids. In: Cobble, J.W. (Ed.), Final Report AP-5111. Electrical Power Research Institute, Palo Alto, CA.

Costa, K.C., Navarro, J.B., Shock, E.L., Zhang, C.L., Soukup, D., Hedlund, B.P., 2009. Microbiology and geochemistry of great boiling and mud hot springs in the United States Great Basin. Extremophiles 13, 447–459.

de Beaumont, E., 1847. Émanations volcaniques et métallifères. Bull. Soc. Geol. Fr. 4 (2nd Series), 1249.

De Leon, K.B., Gerlach, R., Peyton, B.M., Fields, M.W., 2013. Archaeal and bacterial communities in three alkaline hot springs in Heart Lake Geyser Basin, Yellowstone National Park. Front. Microbiol. 4. http://dx.doi.org/10.3389/fmicb.2013.00330.

Dodsworth, J.A., Hedlund, B.P., 2010. Microbiology and geochemistry of Smith Creek and Grass Valley Hot Springs: emerging evidence for wide distribution of novel thermophilic lineages in the U.S. Great Basin. J. Earth Sci. 21, 315–318.

Donahoe-Christiansen, J., D'Imperio, S.D., Jackson, C.R., Inskeep, W.P., McDermott, T.M., 2004. Arsenite-oxidizing *Hydrogenobaculum* strain isolated from an acid-sulfate-chloride geothermal spring in Yellowstone National Park. Appl. Environ. Microbiol. 70, 1865–1868.

Dreyer, R.M., 1940a. The geochemistry of quicksilver mineralization. Econ. Geol. 35, 17–48.

Dreyer, R.M., 1940b. The geochemistry of quicksilver mineralization; part II, petrographic aspects of the geochemistry of quicksilver mineralization. Econ. Geol. 35, 140–157.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983.

Edgcomb, V.P., Molyneaux, S.J., Saito, M.A., LLoyd, K., Boer, S., Wirsen, C.O., et al., 2004. Sulfide ameliorates metal toxicity for deep-sea hydrothermal vent archaea. Appl. Environ. Microbiol. 70, 2551–2555.

Engle, M.A., Gustin, M.S., Goff, F., Counce, D.A., Janik, C.J., Bergfeld, D., et al., 2006. Atmospheric mercury emissions from substrates and fumaroles associated with three hydrothermal systems in the western United States. J. Geophys. Res. 111, D17304. http://dx.doi.org/10.1029/2005JD006563.

Farrell, R.E., Germida, J.J., Huang, P.M., 1990. Biotoxicity of mercury as influenced by mercury(II) speciation Appl. Environ. Microbiol. 56, 3006–3016.

Freedman, Z., Zhu, C., Barkay, T., 2012. Mercury resistance and mercury reductase activities and expression among chemotrophic thermophilic *Aquificae*. Appl. Environ. Microbiol. 78, 6568–6575.

Glew, D.N., Hames, D.A., 1972. Aqueous nonelectrolyte solutions. Part XI. Mercury solubility in 6.10 molal sodium chloride. Can. J. Chem. 50, 3124–3128.

Hidalgo, G., Chen, X., Hay, A.G., Lion, L.W., 2010. Curli produced by *Escherichia coli* PHL628 provide protection from Hg(II). Appl. Environ. Microbiol. 76, 6939–6941.

Horn, J.M., Brunke, M., Deckwer, W.-D., Timmis, K.N., 1994. *Pseudomonas putida* strains which constitutively overexpress mercury resistance for biodetoxification of organomercurial pollutants. Appl. Environ. Microbiol. 60, 357–362.

Hsu-Kim, H., Kucharzyk, K.H., Zhang, T., Deshusses, M.A., 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. Environ. Sci. Technol. 47, 2441–2456.

Hugenholtz, P., Pitulle, C., Hershberger, K.L., Pace, N.R., 1998. Novel division level bacterial diversity in a Yellowstone hot spring. J. Bacteriol. 180, 366–376.

Inskeep, W.P., Rusch, D.B., Jay, Z., Herrgard, M.J., Kozubal, M.A., Richardson, T.H., et al., 2010. Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. PLoS One 5, e9773.

Inskeep, W.P., Jay, Z.J., Tringe, S.G., Herrgard, M.J., Rusch, D.B., Members YMPSCaWG, 2013. The YNP metagenomic project: environmental parameters responsible for microbial distribution in the Yellowstone geothermal ecosystem. Front. Microbiol. 4, 67. http://dx.doi.org/10.3389/fmicb.2013.00067.

Jay, Z.J., 2014. Linking geochemistry with microbial community structure and function in sulfidic geothermal systems of Yellowstone National Park. Land Resources and Environmental Sciences. Ph.D.Montana State University, Bozeman, MT, p. 258

Kato, K., Kobayashi, T., Yamamoto, H., Nakagawa, T., Maki, Y., Hoaki, T., 2014. Microbial mat boundaries between chemolithotrophs and phototrophs in geothermal hot spring effluents. Geomicrobiol J. 21, 91–98.

Kelly, C.A., Rudd, J.W.M., Holoka, M.H., 2003. Effect of pH on mercury uptake by an aquatic bacterium: implications for Hg cycling. Environ. Sci. Technol. 37, 2941–2946.

Klatt, C.G., Inskeep, W.P., Herrgard, M.J., Jay, Z.J., Rusch, D.B., Tringe, S.G., et al., 2013. Community structure and function of high-temperature chlorophototrophic microbial mats inhabiting diverse geothermal environments. Front. Microbiol. 4. http://dx.doi.org/10.3389/fmicb.2013.00106.

Kostal, J., Mulchandani, A., Gropp, K.E., Chen, W., 2003. A temperature responsive biopolymer for mercury remediation. Environ. Sci. Technol. 37, 4457–4462.

Kozubal, M., Macur, R.E., Korf, S., Taylor, W.P., Ackerman, G.G., Nagy, A., et al., 2008. Isolation and distribution of a novel iron-oxidizing crenarchaeon from acidic geothermal springs in Yellowstone National Park. Appl. Environ. Microbiol. 74, 942–949.

Kozubal, M.A., Macur, R.E., Jay, Z.J., Beam, J.P., Malfatti, S.A., Tringe, S.G., et al., 2012. Microbial cycling in acidic geothermal springs of Yellowstone National Park: integrating molecular surveys, geochemical processes, and isolation of novel Fe-active microorganisms. Front. Microbiol. 3. http://dx.doi.org/10.3389/fmicb.2012.00109.

Krup, R., 1988. Physicochemical aspects of mercury metallogenesis. Geochem. J. 69, 345–356.

Kulp, T.R., Hoeft, S.E., Asao, M., Madigan, M.T., Hollibaugh, J.T., Fisher, J.C., et al., 2008. Arsenic(III) fuels anoxygenic photosynthesis in hot springs biofilms from Mono Lake, California. Science 321, 967–970.

Latinwo, L.M., Donald, C., Ikediobi, C., Silver, S., 1998. Effects of intracellular glutathione on sensitivity of *Escherichia coli* to mercury and arsenite. Biochem. Biophys. Res. Commun. 242, 67–70.

Lebedeva, E.V., Alawi, M., Fiencke, C., Namsaraev, B., Bock, E., Spieck, E., 2005. Moderately thermophilic nitrifying bacteria from a hot spring of the Baikal rift zone. FEMS Microbiol. Ecol. 54, 297–306.

Mariner, R.H., Presser, T.S., Evans, W.C., 1977. Hot springs of the central Sierra Nevada, California. Open File Report. U.S. Geological Survey, Menlo Park, CA, p. 27.

Marteyn, B., Sakr, S., Farci, S., Bedhomme, M., Chardonnet, S., Decottignies, P., et al., 2013. The *Synechocystis* PCC6803 MerA-like enzyme operates in the reduction of both mercury and uranium under the control of the glutaredoxin 1 enzyme. J. Bacteriol. 195, 4138–4145.

Mathur, J., Bizzoco, R.W., Ellis, D.G., Lipson, D.A., Poole, A.W., Levine, R., et al., 2007. Effects of abiotic factors on the phylogenetic diversity of bacterial communities in acidic thermal springs. Appl. Environ. Microbiol. 73, 2612–2623.

Meyer-Dombard, D.R., Swingley, W., Raymond, J., Havig, J.R., Shock, E.L., Summons, R.E., 2011. Hydrothermal ecotones and streamer biofilm communities in the Lower Geyser Basin, Yellowstone National Park. Environ. Microbiol. 13, 2216–2231.

Miller, S.R., Stron, A.L., Jones, K.L., Ungerer, M.C., 2009. Bar-coded pyrosequencing reveals shared bacterial community properties along the temperature gradients of two alkaline hot springs in Yellowstone National Park. Appl. Environ. Microbiol. 75, 4565–4572.

Miller-Coleman, R.L., Dodsworth, J.A., Ross, C.A., Shock, E.L., Williams, A., Hartnett, H.E., et al., 2012. Korarchaeota diversity, biogeography,and abundance in Yellowstone and Great Basin hot springs and ecological niche modeling based on machine learning. PLoS One 7, e35964.

Nies, D.H., 1999. Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol. 51, 730–750.

Papke, T.R., Ramsing, N.B., Bateson, M.M., Ward, D.M., 2003. Geographical isolation in hot spring cyanobacteria. Environ. Microbiol. 5, 650–659.

Purcell, D., Sompong, U., Kim, L.C., Barraclough, T.G., Peerapornpisal, Y., Pointing, S.B., 2007. The effects of temperature, pH, and sulfide on the community structure of hyperthermophilic streamers in hot springs of northern Thailand. FEMS Microbiol. Ecol. 60, 456–466.

Ramsing, N.B., Ferris, M.J., Ward, D.M., 2000. Highly ordered vertical structure of *Synechococcus* populations within the one-millimeter-thick photic zone of a hot spring cyanobacterial mat. Appl. Environ. Microbiol. 66, 1038–1049.

Reardon, C.L., Cummings, D.E., Petske, L.M., Kinsall, B.L., Watson, D.B., Peyton, B.M., et al., 2004. Composition and diversity of microbial communities recovered from surrogate minerals incubated in an acidic uranium-contaminated aquifer. Appl. Environ. Microbiol. 70, 6037–6046.

Reysenbach, A.-L., Banta, A., Civello, S., Daly, J., Mitchel, K., Lalonde, S., et al., 2005. Aquificales in Yellowstone National Park. In: Inskeep, W.P., McDermott, T.M. (Eds.), Geothermal Biology and Geochemistry in Yellowstone National Park. Thermal Biology Institute and Department of Land Resources & Environmental Sciences, Bozeman, pp. 129–142.

Romano, C., D'Imperio, S., Woyke, T., Mavromatis, K., Lasken, R., Shock, E.L., et al., 2013. Comparative genomic analysis of phylogenetically closely related *Hydrogenobaculum* sp. isolates from Yellowstone National Park. Appl. Environ. Microbiol. 79, 2932–2943.

Ross, K.A., Feazel, L.M., Robertson, C.E., Fathepure, B.Z., Wright, K.E., Turk-McLeod, R.M., et al., 2012. Phototrophic phylotypes dominate mesothermal microbial mats associated with hot springs in Yellowstone National Park. Microb. Ecol. 64, 162–170.

Ruff-Roberts, A.L., Kuenen, J.G., Ward, D.M., 1994. Distribution of cultivated and uncultivated cyanobacteria and *Chloroflexus*-like bacteria in hot spring microbial mats. Appl. Environ. Microbiol. 60, 697–704.
Sabadell, J.E., Axtmann, R.C., 1975. Heavy metal contamination from geothermal sources. Environ. Health Perspect. 12, 1–7.
Schaefer, J.K., Yagi, J., Reinfelder, J.R., Cardona, T., Ellickson, K.M., Tel-Or, S., et al., 2004. Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury-contaminated natural waters. Environ. Sci. Technol. 38, 4304–4311.
Schelert, J., Dixit, V., Hoang, V., Simbahan, J., Drozda, M., Blum, P., 2004. Occurrence and characterization of mercury resistance in the hyperthermophilic archaeon *Sulfolobus solfataricus* by use of gene disruption. J. Bacteriol. 186, 427–437.
Sharp, C.E., Brady, A.L., Sharp, G.H., Grasby, S.E., Stott, M.B., Dunfield, P.F., 2014. Humbolt's spa: microbial diversity is controlled by temperature in geothermal environments. ISME J. 8, 1166–1174.
Simbahan, J., Kurth, E., Schelert, J., Dillman, A., Moriyama, E., Jovanovich, S., et al., 2005. Community analysis of a mercury hot spring supports occurrence of domain-specific forms of mercuric reductase. Appl. Environ. Microbiol. 71, 8836–8845.
Smith, D.H., 1967. R factors mediate resistances to mercury, nickel, and cobalt. Science 156, 1114–1116.
Spear, J.R., Walker, J.J., Pace, N.R., 2006. Microbial Ecology and Energetics in Yellowstone Hot Springs. vol. 14. Yellowstone Science, pp. 17–24.
Takacs-Vesbach, C., Mitchell, K., Jackson-Weaver, O., Reysenbach, A.-L., 2008. Volcanic calderas delineate biogeographical provinces among Yellowstone thermophiles. Environ. Microbiol. 10, 1681–1689.
Takacs-Vesbach, C., Inskeep, W.P., Jay, Z.J., Herrgard, M.J., Rusch, D.B., Tringe, S.G., et al., 2013. Metagenome sequence analysis of filamentous microbial communities obtained from geochemically distinct geothermal channels reveals specialization of three aquificales lineages. Front. Microbiol. 4. http://dx.doi.org/10.3389/fmicb.2013.00084.
Thompson, J.M., DeMonge, J.M., 1996. Chemical analyses of hot springs, pools, and geysers from Yellowstone National Park, Wyoming, and vicinity, 1980–1993. Open-file Report 96–68. U.S. Geological Survey, Washington, D.C.
Tin, S., Bizzoco, R.W., Kelley, S.T., 2011. Role of the terrestrial subsurface in shaping geothermal spring microbial communities. Environ. Microbiol. Rep. 3, 491–499.
Varekamp, J.C., Bruseck, P.R., 1986. Global mercury flux from volcanic and geothermal sources. Appl. Geochem. 1, 65–73.
Varekamp, J.C., Buseck, P.R., 1983. Hg anomalies in soils: a geochemical exploration method for geothermal areas. Geothermics 12, 29–47.
Varekamp, J.C., Buseck, P.R., 1984. The speciation of mercury in hydrothermal systems, with applications to ore deposition. Geochim. Cosmochim. Acta 48, 177–185.
Vetriani, C., Chew, Y.S., Miller, S.M., Yagi, J., Coombs, J., Lutz, R.A., et al., 2005. Mercury adaptation among bacteria from a deep-sea hydrothermal vent. Appl. Environ. Microbiol. 71, 220–226.
Wagner-Döbler, I., Lünsdorf, H., Lübbehüsen, T., von Canstein, H.F., Li, Y., 2000a. Structure and species composition of mercury-reducing biofilms. Appl. Environ. Microbiol. 66, 4559–4563.
Wagner-Döbler, I., von Canstein, H., Li, Y., Timmis, K.N., Deckwer, W.-D., 2000b. Removal of mercury from chemical wastewater by microorganisms in technical scale. Environ. Sci. Technol. 34, 4628–4634.
Wang, Y., Freedman, Z., Lu-Irving, P., Kaletsky, R., Barkay, T., 2009. An initial characterization of the mercury resistance (mer) system of the thermophilic bacterium *Thermus thermophilus* HB27. FEMS Microbiol. Ecol. 67, 118–129.
Wang, Y., Boyd, E., Crane, S., Lu-Irving, P., Krabbenhoft, D., King, S., et al., 2011. Environmental conditions constrain the distribution and diversity of archaeal *merA* in Yellowstone National Park, Wyoming, U.S.A. Microb. Ecol. 62, 739–752.
Ward, D.M., Ferris, M.J., Nold, S.C., Bateson, M.M., 1998. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol. Mol. Biol. Rev. 62, 1353–1370.
Waring, G.A., 1965. Thermal springs of the United States and other countries of the world; a summary. Professional Paper 492. U.S. Geological Survey, Washington, D.C., p. 383.
Whitaker, R.J., Grogan, D.W., Taylor, J.W., 2003. Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science 301, 976–978.
White, D.E., 1955. Thermal springs and epithermal ore deposits. Econ. Geol. 99–154 (50th Ann).
White, D.E., 1957. Magmatic, connate, and metamorphic waters. Geol. Soc. Am. Bull. 68, 1659–1682.
White, D.E., Hem, J.D., Waring, G.S., 1963. Chemical composition of subsurface waters. Professional Paper 440F. U.S.G.S., Washington, D.C., p. 67.