Mercury (Hg) resistance (mer) by the reduction of mercuric to elemental Hg is broadly distributed among the Bacteria and Archaea and plays an important role in Hg detoxification and biogeochemical cycling. MerA is the protein subunit of the homodimeric mercuric reductase (MR) enzyme, the central function of the mer system. MerA sequences in the phylum Aquificae form the deepest-branching lineage in Bayesian phylogenetic reconstructions of all known MerA homologs. We therefore hypothesized that the merA homologs in two thermophilic Aquificae, Hydrogenobaculum sp. strain Y04AAS1 (AAS1) and Hydrogenivirga sp. strain 128-5-R1-1 (R1-1), specified Hg resistance. Results supported this hypothesis, because strains AAS1 and R1-1 (i) were resistant to >10 μM Hg(II), (ii) transformed Hg(II) to Hg(0) during cellular growth, and (iii) possessed Hg-dependent NAD(P)H oxidation activities in crude cell extracts that were optimal at temperatures corresponding to those strains’ optimal growth temperatures, 55°C for AAS1 and 70°C for R1-1. While these characteristics all conformed with the mer system paradigm, expression of the Aquificae mer operons was not induced by exposure to Hg(II) as indicated by unity ratios of merA transcripts, normalized to gyrA transcripts for hydrogen-grown AAS1 cultures, and by similar MR specific activities in thiosulfate-grown cultures with and without Hg(II). The Hg(II)-independent expression of mer in the deepest-branching lineage of MerA from bacteria whose natural habitats are Hg-rich geothermal environments suggests that regulated expression of mer was a later innovation likely in environments where microorganisms were intermittently exposed to toxic concentrations of Hg.

Microbes must have been exposed to toxic heavy metals since the beginning of life on Earth and have evolved diverse mechanisms to live in the presence of high concentrations of toxic metal ions (42). These mechanisms, such as efflux, intra- or extra-cellular precipitation, and enzyme-mediated transformations, control intracellular concentrations of heavy metal ions that may be inhibitory to physiological functions and form non-specific complex compounds in the cell (28). While much of our existing knowledge of these resistance mechanisms has arisen from research motivated by metal contamination from the perspective of human and environmental health (5, 9, 11, 26, 29), a cosmopolitan distribution of metal-resistant microorganisms inhabiting environments that are enriched with metals of geological origin suggests evolution of metal ion resistance prior to industrial release of metal contaminants (2, 13, 50).

Mercury (Hg) is a potent neurotoxic substance and the heavy metal most toxic to microorganisms due to its high affinity to sulfur (27). Globally distributed Hg (3) is toxic to humans and wildlife, mostly due to the accumulation of methylmercury (MeHg) in aquatic and terrestrial food webs (7). Microbial activities are central in modulating environmental Hg toxicity and mobility. Resistance to inorganic Hg [Hg(II)] is controlled by the activities of the enzyme mercuric reductase (MR), an NAD(P)H-dependent flavin oxidoreductase which catalyzes the reduction of Hg(II) to the elemental form, Hg(0). The gene encoding MR, merA, is part of the Hg resistance (mer) operon, which is widespread among both Bacteria and Archaea (2, 3, 43), allowing these organisms to survive in the presence of elevated Hg concentrations (3, 4). At a minimum, Hg resistance systems are comprised of transport, enzymatic, and regulatory functions. MerT and a number of alternative transporters are involved in the transport of thiocellated Hg(II) into the cytoplasm for reduction by MR (3). MerR regulates expression of the mer operon, binding to the operator/promoter (O/P) region to repress transcription in the absence of Hg(II). When present, Hg(II) binds to the MerR-mer O/P and RNA polymerase complex, prompting the DNA to unwind, inducing transcription of the operon’s functional genes (3, 17).

A recent body of literature supports the hypothesis that microbial resistance to Hg evolved in geothermal environments where microbial life has perhaps been exposed to Hg since the beginning of life on Earth (2, 35, 48). Mercury-resistant microbes were readily isolated from deep-sea hydrothermal vents (48) and terrestrial hot springs (10, 43), and their distribution suggested a role in adaptation to Hg toxicity. Culture-independent techniques detected mer genes in Yellowstone National Park (YNP) (50) and Coso Hot Springs, CA (43). The large-scale sequencing of microbial genomes has resulted in an increased availability of merA sequences and allowed for a robust analysis of gene evolution, further supporting an origin and early evolution of Hg resistance among thermophilic microbes from geothermal environments (2).

To date, functional mer operons have been characterized in mesophilic Actinobacteria, in Firmicutes, among the Beta- and Gammaproteobacteria (2), and in one thermophilic bacterium representing an early bacterial lineage, Thermus thermophilus HB27 (51). The phylum Aquificae contains primary producers which are dominant in many geothermal environments (46) and
represents the deepest-branching bacterial lineage (23). Furthermore, merA homologs in the genomes of Hydrogenobaculum sp. strain Y04AAS1 (AAS1) and Hydrogenivirga sp. strain 128-5-R1-1 (R1-1) (34) form the deepest-branching lineage in a MerA phylogeny (2). Strain AAS1 was isolated from a small channel proximal to Obsidian Pool Prime, YNP, and R1-1 was isolated from the Eastern Lau Spreading Center, South Pacific (34), both of which are geothermal environments similar to those where elevated Hg concentrations were reported (12, 21, 31). The basal position of the Aquificae loci in the MerA phylogenetic reconstructions suggests that merA originated in an ancestor common to deep-branching thermophilic bacteria. Here, we report on the activity and characteristics of the Hg resistance systems of two Aquificae strains representing chemotrophic primary producers in many geothermal environments.

MATERIALS AND METHODS

MerA phylogeny and bioinformatic analyses. MerA sequences were compiled in April 2011 by tblastx searches of the Entrez Protein database (http://www.ncbi.nlm.nih.gov/sites/entrez/db=protein) using the MerA amino acid sequences of Tsu01 (accession number CA77323.1) and Salilbolasus salticarius P2 (AAK42805.1) as queries. These searches identified two open reading frames (ORFs), HYO4AAS1_1213 and H12825_05690, as merA homologs in Hydrogenobaculum sp. Y04AAS1 and Hydrogenivirga sp. 128-R1-1, respectively. The alignment block of bacterial and archaeal MerA sequences, corresponding to positions 8 to 472 of Streptomyces lividans (P30341), was used in a Bayesian inferred phylogenetic reconstruction, performed as described by Wang et al. (50). Of the 284 gene homologs available, 99 were selected for reconstruction; the selected homologs represented all major clusters in the MerA phylogeny. Putative promoter regions were determined using the BPROM tool (Softberry Inc., Mt. Kisco, NY).

Bacterial strains and growth conditions. Strains Hydrogenobaculum sp. Y04AAS1 (AAS1), Hydrogenivirga sp. 128-5-R1-1 (R1-1), and Persephonella marina EX-H1 were generously provided by Anna-Louise Rey- senbach (Portland State University); major characteristics of these strains are summarized in Table S1 in the supplemental material. All growth media were prepared under a CO2 headspace, microaerophilic conditions are summarized in Table S1 in the supplemental material. All growth media were prepared under a CO2 headspace, microaerophilic conditions. Growth measurements.

Production of Hg(0) by growing cultures. Cultures were grown with 203HgCl2 as described above to stationary phase when the headspace of the incubation vessel was flushed with sterile air for 40 min to drive 203Hg(0) that accumulated during growth into a Hg-trapping solution consisting of 0.75% KMnO4, 0.40% K2S2O3, 3.5% HNO3, and 5% H2SO4. At the conclusion of the 40 min, the remaining growth medium was acidified to 0.5 N HCl and mixed by vortexing. Aliquots of 250 μl were removed from the acidified stationary-phase culture and the trapping solution for scintillation counting. Control treatments included autoclaved cells and un inoculated media. Significance (P < 0.05) of differences in Hg(II) loss rates for each treatment was calculated using Student’s t test.

Mercuric reductase (MR) assays. Mid-log-phase cultures of Hydrogenobaculum sp. Y04AAS1 and Hydrogenivirga sp. 128-R1-1 were diluted 100-fold into 250 ml of medium in rubber-capped 2-liter Pyrex medium bottles (Corning, Lowell, MA) with a microaerophilic headspace as described under “Bacterial strains and growth conditions” above. Cultures were grown to mid-log phase, and cells were harvested by centrifugation for 10 min at 5,750 X g at 4°C in a prerefrigerated Sorvall RC-5B centrifuge (Thermo Scientific, Waltham, MA). Pelleted cells were washed in phosphate-buffered saline and stored at −20°C until used for further analysis.

Cell-free lysates were prepared by following protocols described by Vetriani et al. (48). Cells were resuspended to a concentration of 200 mg ml−1 (wet weight) in a buffer consisting of 20 mM sodium phosphate (pH 7.5), 0.5 mM EDTA, and 1 mM β-mercaptoethanol and were lysed by
RESULTS AND DISCUSSION
Identification of putative mer operons in the genomes of Hydrogenobaculum sp. Y04AAS1 and Hydrogenivirga sp. 128-5-R1-1.

An updated phylogeny that included all MerA homologs in databases as of April 2011 confirmed (2) the basal position of the *Aquificae* sequences in a sister position to all archaeal sequences with posterior probability values of 100. This cluster shared a common, likely bacterial, ancestor with a large cluster consisting of the remaining bacterial MerA sequences (see Fig. S1 in the supplemental material). These results highlight the important position of the *Aquificae* MerA, potentially representing an ancestral state of the protein among all prokaryotes.

The MerA sequences of both *Aquificae* loci contained signature motifs known to be required for MerA activity (Fig. 1A) (3). In *Hydrogenobaculum* sp. Y04AAS1, MerA is 464 amino acids (aa) long, while in *Hydrogenivirga* sp. 128-5-R1-1, it is 545 residues long due to the presence of NmerA, the ~70-aa N-terminal extension heavy metal-associated (HMA) domain that is a part of more than half of all MerA sequences (2). Mercuric reductase activities were documented for both variants of MerA (51), and NmerA is not essential for MerA activity (22).

In both *Aquificae* strains, two ORFs upstream of the putative merA code for proteins that bear homology to other Mer functions (Fig. 1B). The merA-proximal ORFs, loci YP_002121875 (78 aa) and ZP_021777251 (92 aa) in the genomes of strains AAS1 and R1-1, respectively, may encode the Hg-scavenging protein MerP, as they include the signature metal binding motif sequence GCTCxxC (Fig. 1A). However, the ~19-residue Sec-type signal known to direct proteobacterial MerP to the periplasmic space (3, 20) is missing in both *Aquificae* loci, leaving in question the transport of their gene products to the periplasmic space and thus their function as MerP.

ORFs encoding MerT homologs are found upstream of the putative merP genes in both genomes (loci HY04AAS1_1211 and ZP_021777250 [Fig. 1A]). Homology includes three hydrophobic inner membrane-spanning sequences as determined by TMpred (18), the vicinal C pair in the first membrane-spanning sequence, and a C pair located in the cytoplasmic loop between the second and the third membrane-embedded sequences (36).

Homologs of the mer operon regulator, MerR, were not found in the genomes of strains AAS1 and R1-1, though several homologs of ArsR/SmtB, a regulator reported in *mer* systems of the *Archaea* and the *Actinobacteria* (2, 3), were identified using tBLASTn searches. Based on homology searches, putative promoter regions (33) were identified 26 and 15 bp upstream of the ATG start codon of the ORFs preceding *merT* in strains *Hydrogenobaculum* sp. Y04AAS1 and *Hydrogenivirga* sp. 128-5-R1-1, respectively. These included ~35 and ~10 regions (separated by 16 and 17 nucleotides, rather than by 19 nucleotides as is common to promoters that are regulated by MerR [8]), Shine-Dalgarno ribosomal binding sites, and translation initiation signals. No other possible promoter sequences were identified upstream of any of the mer homologs of these strains.

Mercury resistance in *Hydrogenobaculum* sp. AAS1, *Hydrogenivirga* R1-1, and *Persephonella marina* EX-H1.

The technology to genetically manipulate bacteria that belong to the *Aquificae* is not yet available, and thus, obtaining merA mutants of strains AAS1 and R1-1 was not possible. We therefore compared Hg tolerance in strains AAS1 and R1-1 to that of *P. marina* EX-H1 as a
control. Mercury forms complexes with medium components, which control its bioavailability and therefore toxicity (14). Employed growth media included either S\(\text{O}_3^2\)/H\(\text{H}_2\)O as a sole energy source, leading to very different Hg(II) speciations. MINEQL+ calculations revealed that in the presence of 10 \(\text{mM}\) HgCl\(_2\) and 8 \(\text{mM}\) S\(\text{O}_3^2\)/H\(\text{H}_2\)O, Hg(II) speciated as negatively charged Hg-S\(\text{O}_3\) complexes, with 84% as Hg(S\(\text{O}_3\))\(_2^2\)/H\(\text{H}_2\)O and 16% as Hg(S\(\text{O}_3\))\(_3^4\)/H\(\text{H}_2\)O (see Table S4 in the supplemental material). In the H\(\text{H}_2\) growth media, Hg speciated as uncharged HgCl\(_2\) and HgCl(OH) complexes. The model suggested similar Hg speciation in the range of 2 to 60 \(\text{mM}\) added HgCl\(_2\) (data not shown).

Using H\(\text{H}_2\) and S\(\text{O}_3^2\)/H\(\text{H}_2\)O as electron donors, the lowest tested HgCl\(_2\) concentrations, 2 \(\text{mM}\) and 5 \(\text{mM}\), respectively, completely inhibited growth of the control \(P.\) marina EX-H1, while Hydrogenobaculum sp. Y04AAS1 (AAS1) and Hydrogenivirga sp. 128-R1-1 (R1-1), with putative Mer operons had a higher tolerance to Hg than the control strain, it was impossible to quantitatively compare their responses or to assess the effects of electron donor and Hg speciation on the level of Hg resistance due to variable Hg loss from uninoculated S\(\text{O}_3^2\)/H\(\text{H}_2\)O-amended media (see Fig. S3 in the supplemental material; see also below).

Loss of Hg(II) during growth of strains AAS1 and R1-1. To determine if growth of strains AAS1 and R1-1 was related to the removal of Hg(II) from growth media, changes in Hg(II) concentrations were monitored during growth to stationary phase (Fig. 2). Growth experiments were performed using 5 or 10 \(\mu\text{M}\) HgCl\(_2\) in media containing H\(\text{H}_2\) or S\(\text{O}_3^2\)/H\(\text{H}_2\)O, respectively.

**FIG 1** (A) Alignment of putative Mer proteins, including MerA, MerT, and MerP from *Hydrogenobaculum* sp. Y04AAS1 (AAS1) and *Hydrogenivirga* sp. 128-R1-1 (R1-1), with *mer* sequences from *Pseudomonas aeruginosa* Tn501 as a reference. Highlighted conserved regions of functional importance (3) are as follows: for MerA, the redox active site, two downstream tyrosines, and the carboxy terminus-vicinal CC pair; for MerT, the conserved C residues in the first membrane-spanning loop and in the cytoplasmic loop between the second and third membrane-spanning domains; and for MerP, the metal binding motif GTMxxC. The membrane-spanning domains (determined using TMpred [http://www.ch.embnet.org/software/TMPRED_form.html]) are boxed (numbered 1 to 3) in MerT. Gray circles indicate the carboxy termini of the proteins. Numbering indicates position in the Tn501 sequence. (B) *mer* operon gene order. Arrowed boxes indicate each ORF and the direction of transcription; accession numbers are included above each box. Names of putative gene products and corresponding numbers of amino acids (AA) are given above the boxes. The numbered line below each ORF represents the nucleotide position marking the start of the gene counted from the transcription start nucleotide upstream of the hypothetical proteins in each operon.
Loss of $^{203}$Hg(II) from all growth media occurred well before the commencement of growth by strains R1-1 and AAS1. Initial rates of Hg loss from inoculated media were calculated after subtracting loss from uninoculated controls (see below). For strain AAS1, loss was approximately 25-fold faster when strains were grown on $\text{S}_2\text{O}_3^{2-}$ rather than $\text{H}_2$, with initial rates of Hg(II) loss of $125.5 \pm 34.1$ and $5.7 \pm 0.03$ μmol Hg(II) lost $\text{h}^{-1} \cdot 10^6 \text{cells}^{-1}$, respectively ($P < 0.02$ [Fig. 2D]). This is not surprising considering the higher energy yield from the oxidation of $\text{S}_2\text{O}_3^{2-}$ ($\Delta G^o = -818.3 \text{kJ}/\text{reaction}$) than from that of $\text{H}_2$ ($\Delta G^o = -237 \text{kJ}/\text{reaction}$) and the dependency of Hg(II) reduction by MR on availability of reducing equivalents (4). Strain R1-1 lost Hg(II) at a rate of $150.0 \pm 0.01$ μmol Hg(II) $\text{h}^{-1} \cdot 10^6 \text{cells}^{-1}$, statistically indistinguishable from the rate of strain AAS1 grown on $\text{S}_2\text{O}_3^{2-}$. Interestingly, more Hg was lost from the heat-killed controls of strain R1-1 than from the uninoculated controls ($P < 0.02$) at a rate that was slightly, though significantly ($P = 0.05$), higher. Since autoclaving (105°C for 30 min) resulted in cell death, as indicated by failure to grow following such treatment (data not shown), the MR of strain R1-1 must be highly tolerant to heat.

In the $\text{S}_2\text{O}_3^{2-}$-amended media, significant amounts of $^{203}$Hg(II) were lost ($P < 0.05$) from the heat-killed and uninoculated controls (see Fig. S3 in the supplemental material) that were incubated at 55°C (strain AAS1 controls [Fig. 2A]) and 70°C (strain R1-1 controls [Fig. 2B]). At the commencement of growth, medium-only controls of strain AAS1 (at >40 h postinoculation) and R1-1 (at >20 h) had only $4.8 \pm 1.3$ and $5.4 \pm 1.4$ μmol Hg(II) remaining in the media, respectively, approximately half of the starting concentration. No such loss was observed in the $\text{H}_2$-supplemented medium. Since loss did not occur when $\text{S}_2\text{O}_3^{2-}$-medium-only controls were incubated at room temperature (data not shown), this abiotic loss resulted from elevated temperatures. This suggests that uncharged Hg(II) complexes are less volatile at elevated temperatures than the charged Hg-$\text{S}_2\text{O}_3^{2-}$ complexes. Nevertheless, the live growing cultures of both strains lost a significantly greater proportion of $^{203}$Hg(II) than all abiotic controls ($P < 0.05$).

The Hg concentration in $\text{H}_2$-amended AAS1 cultures initially declined, then increased at 20 h following inoculation, and then remained stable at about 3 μM (Fig. 2C). Since incubations were carried out in closed systems where the formed gaseous Hg(0) likely partitioned between the medium and the headspace according to Henry’s coefficient (at 35°C, $k_{Hg} = 0.4$ dimensionless [1]), it is possible that some of the initially formed Hg(0) was oxidized to Hg(II) by the $\text{H}_2$-metabolizing culture. Bacterial Hg(0) oxidation has been reported for Escherichia coli, Bacillus subtilis, and Streptomyces venezuelae and attributed to the enzymes catalase and hydperoxidase (45). The results clearly show that cultures of Hydrogenivirga sp. 128-R1-1 and Hydrogenobaculum sp. Y04AAS1 removed Hg from their growth media and that most of this activity took place during the lag phase of growth.

Production of Hg(0) by growing cultures of AAS1 and R1-1. Endpoint mass balance experiments determined that the Hg(II) which was lost during growth of strains AAS1 and R1-1 was converted to Hg(0) (Table 1). When grown to early stationary phase on $\text{S}_2\text{O}_3^{2-}$, strains AAS1 and R1-1 produced $3.8 \pm 2.4$ and $3.1 \pm 0.02$ μmol Hg(II)/h/$10^6$ cells. Inoculated cultures of strain AAS1 lost Hg(II) at a rate that was significantly higher than that observed in uninoculated controls ($P < 0.05$). All data represent the mean Hg(II) loss of triplicate growing cultures ± 1 standard deviation (SD) after subtracting loss rates of uninoculated controls.
1.3 μmol Hg(0), respectively. When grown on H₂, strain AAS1 produced 2.2 ± 0.8 μmol Hg(0), statistically similar to the amount produced by S₂O₃²⁻-grown cultures. Production of Hg(0) by heat-killed controls was approximately 10-fold lower than that by growing cultures for all treatments. Mass balance calculations showed recoveries of 85 to 122% of the added ²⁰³HgCl₂.

MR activities by crude cell extracts of strains AAS1 and R1-1. Specific rates of MR activity were measured to determine this enzyme’s role in the formation of Hg(0) by Hydrogenobaculum sp. Y04AAS1 and Hydrogenivirga sp. 128-5-R1-1. Preliminary experiments indicated a preference for NADH by R1-1 and for NADPH by AAS1 crude cell extracts (data not shown), and therefore all further experiments were performed with each strain’s preferred reductant. Given the developing understanding of the importance of mer in thermophilic microbes (2, 40, 51), MR activities of strains AAS1 and R1-1 were determined at a range of temperatures. Results showed a correspondence between optimal temperatures for growth and for Hg-dependent NAD(P)H oxidation with maximum apparent specific MR activity for AAS1 (37.7 ± 1.1 μM mg protein⁻¹) at 50°C and for R1-1 (3.2 ± 0.2 μM mg protein⁻¹) at 70°C (Fig. 3). Strain AAS1’s MR was active at a temperature range of 30 to 70°C and that of strain R1-1 was active from 60 to 87°C, and both extracts had very low activities below 40°C. MR activity was not detected in crude extracts of the control strain P. marina EX-H1 when tested at its optimal growth temperature, 70°C. These results suggest that strains AAS1 and R1-1 possessed thermostable MR.

Induction of merA transcription and MR activity. mer operon expression is regulated in both Bacteria (47) and Archaea (39) by the regulatory protein MerR and, less frequently, by ArsR/SmtB-like regulators; most mer operons code for these regulators (3). Because gene homologs of these regulators were not found in proximity to merA of strains AAS1 and R1-1, we determined the effects of growth in the presence of HgCl₂ on levels of merA transcription fold induction in strain AAS1 (●) grown on H₂ compared with induction of merA of HB27 (○) and Tn501 (△). Fold induction was calculated as described in Materials and Methods, except that merA expression in Tn501 and HB27 was normalized to expression of 16S rRNA genes rather than to gyrA (data from reference 51). (B) Effect of growth in the presence of Hg on MR specific activities in crude cell extracts of strains AAS1 and R1-1. Cell extract activities were determined for cultures grown with (filled column) or without (clear column) 10 μM HgCl₂ in S₂O₃²⁻-amended media. The means ± 1 SD of triplicate determinations are shown. The same letters above columns indicate no significant difference by Student’s t test (P > 0.05).

FIG 3 Effect of temperature on MR activities. Specific activities of crude cell extracts were determined for Hydrogenobaculum sp. Y04AAS1 (●) and Hydrogenivirga sp. 128-5-R1-1 (○). Activities of R1-1 extracts are expressed as those measured, multiplied by 10. Previously reported data for T. thermophilus (○) and Tn501 (△) (51) are included for comparison. Averages of three to five replicate assays ± 1 SD are presented. One unit of MR activity = 1 μmol NAD(P)H oxidized min⁻¹.

FIG 4 merA expression in Hydrogenobaculum sp. Y04AAS1 and Hydrogenivirga sp. 128-5-R1-1. (A) HgCl₂-dependent (1 μM) transcription fold induction in strain AAS1 (●) grown on H₂ compared with induction of merA of HB27 (○) and Tn501 (△). Fold induction was calculated as described in Materials and Methods, except that merA expression in Tn501 and HB27 was normalized to expression of 16S rRNA genes rather than to gyrA (data from reference 51). (B) Effect of growth in the presence of Hg on MR specific activities in crude cell extracts of strains AAS1 and R1-1. Cell extract activities were determined for cultures grown with (filled column) or without (clear column) 10 μM HgCl₂ in S₂O₃²⁻-amended media. The means ± 1 SD of triplicate determinations are shown. The same letters above columns indicate no significant difference by Student’s t test (P > 0.05).

TABLE 1 Reduction of Hg(II) to Hg(0) by Aquificae cultures

<table>
<thead>
<tr>
<th>Strain</th>
<th>Electron donor</th>
<th>Treatment</th>
<th>Medium Hg (μM)¹</th>
<th>Headspace Hg (μM)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS1</td>
<td>H₂</td>
<td>Growing cultures</td>
<td>3.91 ± 0.18₃</td>
<td>2.19 ± 0.79⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat killed</td>
<td>4.32 ± 0.80⁵</td>
<td>0.21 ± 0.06⁶</td>
</tr>
<tr>
<td></td>
<td>S₂O₃²⁻</td>
<td>Growing cultures</td>
<td>5.02 ± 1.47⁷</td>
<td>3.80 ± 2.38⁸</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat killed</td>
<td>10.87 ± 2.60⁹</td>
<td>0.34 ± 0.03⁹</td>
</tr>
<tr>
<td>R1-1</td>
<td>S₂O₃²⁻</td>
<td>Growing cultures</td>
<td>6.69 ± 0.48¹⁰</td>
<td>3.09 ± 1.34¹¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat killed</td>
<td>8.13 ± 0.48¹²</td>
<td>0.32 ± 0.01¹³</td>
</tr>
</tbody>
</table>

¹ Different letters indicate statistical significance (P < 0.05) by Student’s t test.
² HgCl₂ was added to initial concentrations of 5 and 10 μM to H₂- and S₂O₃²⁻-containing growth media, respectively.

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mer systems (3), expression of mer in strains AS1 and R1-1 was not induced by exposure to Hg(II). The mer operon of the plasmid pMERPH does not include an adjacent merR, though its expression is inducible, likely associated with a regulatory gene located elsewhere on the plasmid (29a). A constitutively expressed Hg-dependent NADH oxidation was recently described for the marine methylotroph *Methylococcus capsulatus* (Bath) (5a). Mercury-independent expression of mer among the *Aquificae* may not be surprising considering that members of this phylum often occupy sulfide-rich geothermal environments (19) where Hg levels were reported at nanomolar to micromolar concentrations (21, 50). Obviously, an elaborate regulatory system would be superfluous in environments with persistent exposure to Hg.

**Evolutionary implications.** Phylogenetic reconstructions consistently place *Aquificae* sequences in a basal position to all bacterial and archaeal MerA proteins (see Fig. S1 in the supplemental material) (2). Thus, our results suggest that the expression of the ancestral Hg detoxification system, which likely originated among thermophilic bacteria in geothermal environments, is not regulated by exposure to Hg. If so, Hg-dependent regulation of this system was likely acquired later, possibly among organisms only intermittently exposed to toxic levels of Hg. We have previously pointed out the increased number of *mer* functions with MerA evolution (2); the present study further clarifies this system’s evolutionary path from simple operons, whose expression does not depend on exposure to Hg, to more complex and finely regulated systems (9a, 17, 45a).

Furthermore, the correspondence of optimal growth and MR activity temperatures of the two thermophilic *Aquificae* strains (Fig. 3) supports the conclusion that *merA* originated among thermophiles (2, 51). In contrast, the optimal temperature of *Thermococcus*’s enzyme was >20°C higher than the optimal growth temperature of *Pseudomonas aeruginosa* (Fig. 3), the mesophilic Hg-resistant bacterium from which *Thermococcus* was isolated (46a). It has been previously proposed that this discrepancy between the optimal growth and enzyme activity temperatures was a relic of MR evolution in high-temperature environments (51). Our results, therefore, strengthen this conclusion and the identification of high-temperature environments as the place for the origin and early evolution of the microbial Hg detoxification system (2, 48, 51).

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**References.**

25. Reference deleted.
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32. Reference deleted.
44. Reference deleted.