Rapid Arsenite Oxidation by *Thermus aquaticus* and *Thermus thermophilus*: Field and Laboratory Investigations

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Thermus aquaticus and *Thermus thermophilus*, common inhabitants of terrestrial hot springs and thermally polluted domestic and industrial waters, have been found to rapidly oxidize arsenite to arsenate. Field investigations at a hot spring in Yellowstone National Park revealed conserved total arsenic transport and rapid arsenite oxidation occurring within the drainage channel. This environment was heavily colonized by *Thermus aquaticus*. In laboratory experiments, arsenite oxidation by cultures of *Thermus aquaticus* YT1 (previously isolated from Yellowstone National Park) and *Thermus thermophilus* HB8 was accelerated by a factor of over 100 relative to abiotic controls. *Thermus aquaticus* and *Thermus thermophilus* may therefore play a large and previously unrecognized role in determining arsenic speciation and bioavailability in thermal environments.

Introduction

Arsenic is a common trace constituent of geothermal fluids with typical concentrations of 1−10 mg L^{-1} (*1*). The natural discharge of these fluids often results in increased arsenic levels in surface waters and aquifers surrounding hot springs (*2*). For example, at Yellowstone National Park, over 100 000 kg of geothermally derived arsenic is estimated to leave the western boundary each year, affecting water quality within a large region (*3*).

Geochemists have long been studying the behavior of As in geothermal systems (*4*). Since the discovery of hyperthermophilic microorganisms, biochemists and microbial ecologists have also been investigating hot springs to explore the diversity and physiology of extremophiles and to seek

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biomolecules with potential technological importance (*5*). The *Thermus* species in particular have been studied extensively over the last three decades in pursuit of novel enzymes and biochemical pathways for industrial applications (*6*). Recently, investigations have begun to address the ecological role of the *Thermus* species (*7*). However, their interactions with the arsenic-rich fluids in which they often live has notably been overlooked.

Microbes are known to play a significant part in As cycling. Microorganisms can impact the mobility of arsenic through indirect natural processes such as oxidative sulfide mineral dissolution (*8*), reduction of iron oxides (*9, 10*), and sulfate reduction (*11, 12*). Direct microbial processes including arsenate (As(V)) respiration (*13*) and arsenite (As(III)) oxidation (*8*) can impact arsenic abundance and speciation as well. Arsenite oxidation by microorganisms is potentially quite significant, as this reaction is kinetically hindered and extremely slow in the absence of a biological or surface catalyst (*14, 15*).

Since the first report in 1918 of arsenite oxidation by bacteria (*8*), a small number of microorganisms having this metabolic capability have been isolated. These include the heterotrophs *Pseudomonas putida* (*16*) and *Alcaligenes faecalis* (*17*) as well as the chemolithoautotrophic arsenite-oxidizers *Pseudomonas arsenitoxidans* (*18*) and "NT-26" (*19*). While arsenite oxidation by these isolates has been studied in the laboratory, their impact on As cycling in nature has not been investigated and remains unknown.

Previous studies of arsenic oxidation in geothermal systems include the work of Stauffer et al. (*20*) in which observations were made indicating rapid oxidation of As(III) to As(V) in hot spring drainage waters of Yellowstone National Park. Microbial activity was implicated in the observed rapid As oxidation. However, biological characterizations were not conducted. A report by Wilkie and Hering (*21*) showed microbial oxidation of arsenic in a stream affected by geothermal fluids. In this system, an unidentified consortium of microorganisms associated with plants was shown to oxidize arsenic at temperatures near 25 °C.

For this study, we have focused on two species: *Thermus aquaticus* and *Thermus thermophilus*. Both organisms are heterotrophic with growth ranges of 40−79 °C and 47−85 °C, respectively (*22, 23*). The *Thermus* species are known to be very widespread, inhabiting nearly all types of thermally influenced circumneutral waters that have been examined, including terrestrial hot springs as well as domestic and industrial sources (*24, 6*). Due to the prevalence of *Thermus aquaticus* and *Thermus thermophilus*, we have combined field and laboratory investigations to assess the affect of these organisms on As cycling in the environment.

Materials and Methods

Laboratory Experiments. *Bacterial Strains and Growth Conditions.* The strains *Thermus aquaticus* YT1 (DSM 625) and *Thermus thermophilus* HB8 (DSM 579) were purchased from the German Collection of Microorganisms and Cell Cultures. Growth medium contained 0.2% (w/v) yeast extract, 0.8 g L^{-1} of $(NH_4)_2SO_4$, 0.4 g L^{-1} of KH_2PO_4, 0.18 g L^{-1} of $MgSO_4 \cdot 7H_2O$, and 1.75 g L^{-1} of NaCl adjusted to pH 7.5 at room temperature with NaOH and autoclaved. When required, 2X (double the concentration of constituents) growth medium was diluted to 1X with autoclaved deionized water and a stock of filter-sterilized 3750 mg L^{-1} of As(III) (arsenious acid; LabChem Inc.) adjusted to pH 7.5 with NaOH. Cultures of *T. aquaticus* and *T. thermophilus* were maintained in the presence of 75 mg L^{-1} arsenite and washed twice with fresh

growth medium prior to culture inoculations. All culturing was carried out using tightly sealed 125 mL screw-cap polycarbonate flasks to prevent evaporation.

Arsenite Oxidation Assay. To test for the ability to oxidize arsenite, *T. aquaticus* and *T. thermophilus* were inoculated into 60 mL of growth medium containing 75 mg L^{-1} of arsenite and incubated at 70 °C with 125 rpm shaking. Control experiments using noninoculated, sterile media with 75 mg L^{-1} of arsenite were also incubated under the same conditions. The high initial level of arsenite used in these experiments was to better facilitate rate calculations and to conform to analytical constraints. One milliliter samples from biological and abiotic experiments were taken over time for measurements of cell density and for determinations of arsenic speciation. Optical density was measured at 600 nm using a Perkin-Elmer Lambda 3 UV/vis spectrophotometer. Samples were centrifuged and decanted, acidified by adding concentrated trace metal-grade HCl to 1% (v/v), and stored at 4 °C for less than 7 days prior to arsenic analyses. Measurements of arsenic speciation in laboratory experiments followed the ion-paired chromatography method of Bushee et al. (*25*) and the arsine generation protocol of Howard and Hunt (*26*). The atomic absorption of arsine was detected at 193.7 nm using a Unicam 969 flame atomic absorption spectrometer.

Field Studies. *Sample Collection.* Fieldwork was carried out over a 2-day period in September 2000 at the Twin Butte Vista Hot Spring in the Lower Geyser Basin of Yellowstone National Park (YNP). Five sampling stations were designated at intervals along the main overflow channel spanning an approximately 18.5 m distance. Biological samples were collected using sterile forceps, placed in 15 mL screw-cap Falcon tubes containing 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS; 8.0 g L^{-1} of NaCl, 0.2 g L^{-1} of KCl, 1.44 g L^{-1} of $Na_2HPO_4 \cdot 7H_2O$, 0.24 g L^{-1} of KH_2PO_4, pH 7.2), and kept on ice. Within 8 h of collection, samples were centrifuged, washed once with cold PBS, and resuspended in ethanol:PBS (1:1; v/v). Fixed biological samples were kept on ice during transport and stored at −20 °C in the laboratory.

Three sets of water samples were collected by syringe from each station and were filtered (0.2 μm Pall Acrodisc) into high-density polyethylene screw-cap bottles. Samples for As and Fe speciation determinations were acidified by 1% (v/v) additions of concentrated trace metal-grade HCl. Samples for cation measurements were acidified by 1% (v/v) additions of electronic-grade HNO_3. The final set of water samples was left unacidified for anion analyses.

Determinations of Inorganic Constituents in Environmental Samples. All reagents were of purity at least equal to the reagent-grade standards of the American Chemical Society. Doubly distilled deionized water and redistilled acids were used in all preparations. United States Geological Survey standard reference water samples were used as independent standards. Samples were diluted as necessary to bring the analyte concentration within the optimal range of the method. For elemental analyses, several dilutions of each sample were analyzed to check for concentration effects on the analytical method. Spike recoveries were also performed on several samples.

Concentrations of major cations and trace metals were determined using a Leeman Labs − DRE inductively coupled plasma optical-emission spectrometer. Major cations were analyzed using the radial view, while the axial view was used for trace metals. As(III) and total As concentrations in the environmental samples were determined using a flow injection analysis system for the generation of arsine and detection using atomic absorption spectrometry (Perkin-Elmer -Analyst 300) (*27*). Levels of As(V) were calculated as the difference between total arsenic and As(III). Fe(II/III) redox species were determined using a modification of the

FerroZine colorimetric method (*28, 29*). Concentrations of major anions were determined chromatographically (*30*) using a Dionex 2010i ion chromatograph with 10-μL and 50-μL sample loops. Alkalinity (as HCO_3^-) was determined using an Orion 960 autotitrator and standardized H_2SO_4 (*31*). Specific conductance was measured using a Orion conductivity meter (model 126).

Field Geochemical Analyses. Measurements of pH, Eh, and water temperature were made in the field using an Orion 290A portable meter and Orion 9107 pH/temperature and Orion 9678 redox electrodes. The pH electrode was calibrated with pH 4, 7, and 10 standard buffers (Fisher) heated to sample temperature by immersion of the buffer vials in the hot spring waters where sampling was performed. Preparation of the ZoBell's solution to calibrate the platinum electrode for Eh measurements and the values for the standard half-cell potentials used in calculating sample Eh are after the method of Nordstrom and Wilde (*32*). The ZoBell's solution was prepared immediately prior to use and brought to sample temperature by immersion of the sealed solution vial in the hot spring fluids before calibration of the meter. Sulfide was measured in the field using a Hach DR/2010 portable datalogging spectrophotometer after Hach method #8131 (Methylene Blue colorimetric analysis equivalent to United States Environmental Protection Agency method 376.2).

Fluorescence in-Situ Hybridizations (FISH). The 16S rRNA-targeted oligonucleotide probes Eub338 (*33*), Arch915 (*34*), S-G-Thus-0438-a−A-18 (*35*), Taq1258 (*36*), and Tth 1258 (*36*) were used in this study. Probes were synthesized and labeled with fluorescein (Eub338 and Arch915) or Cy3 (Thus0438, Taq1258, and Tth 1258) by the University of Wisconsin Biotechnology Center.

Hybridizations were performed according to the protocol of Bond and Banfield (*37*). Fixed environmental samples were homogenized by rigorous vortexing and spotted to gelatin-coated multiwell slides. The organisms *Thermus aquaticus* YT1, *Thermus thermophilus* HB8, *Pseudomonas putida*, *Thermoplasma acidiphilum*, and *Sulfolobus sulfataricus* were fixed and used as controls during the hybridizations. The hybridization buffer contained 20% formamide and each well was probed with Arch915 and Eub338, plus either Thus0438, Taq1258, or Tth1258.

Samples were examined using a Leica LEITZ DMRX epifluorescence microscope equipped with Chroma Technology filter sets 41007a for detection of Cy3 and 41001 for detection of fluorescein. The percentages of hybridized cells were quantified by comparing the total number of cells in a field of view labeled with the Arch915 and Eub338 probes relative to the number of cells labeled with one of the Thus0438, Taq1258, or Tth1258 probes. For stations 2−5, a minimum of 3000 cells in at least six separate wells were counted for each sample; 697 cells in four separate wells were counted for station 1.

Results

Culturing Experiments. Laboratory experiments conducted to examine *Thermus aquaticus* for the ability to oxidize arsenite to arsenate showed that within 12 h after inoculation, arsenite oxidation was accelerated relative to abiotic controls (Figure 1A). A lag period of slow oxidation during the first 16 h of incubation was followed by rapid arsenite oxidation coinciding with the exponential phase of growth. Within 24 h, 100% of arsenite was oxidized to arsenate by *T. aquaticus* at a rate of 0.14 mg L^{-1} min^{-1} during exponential growth. *Thermus thermophilus* showed similar results (Figure 1B) also oxidizing arsenite at a rate of 0.14 mg L^{-1} min^{-1} during exponential growth. In each of the abiotic control experiments, only about 5% of the arsenite was oxidized after 48 h (Figure 1) at an average rate of 0.001 mg L^{-1} min^{-1} ($n = 3$; standard deviation $= 3.6 \times 10^{-4}$).
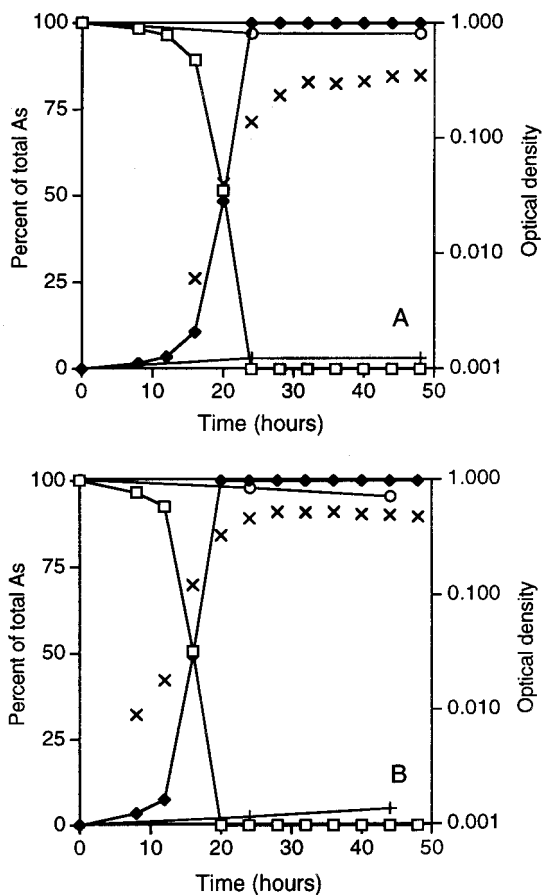
FIGURE 1. Arsenic speciation and culture density during arsenite oxidation by *Thermus aquaticus* YT1 (A) and *Thermus thermophilus* HB8 (B). Linear plot: □, arsenite; ◆, arsenate; ○, arsenite-abiotic control; +, arsenate-abiotic control. Logarithmic plot: ×, optical density of culture.

To confirm arsenite was oxidized through the metabolic activity of *T. aquaticus* and *T. thermophilus*, culturing experiments were carried out in which growth was inhibited by antibiotics (Figure 2). Due to the mode of action of kanamycin and ampicillin, which is to respectively inhibit protein synthesis and prevent cell wall production, the cultures had a limited capacity to oxidize arsenite during the treatment. *T. thermophilus* in particular oxidized arsenic up to 40 h following the addition of the antibiotics. However, the rate of arsenic oxidation by antibiotic-treated cells of both organisms was significantly reduced relative to untreated cells.

Additional experiments were conducted to ascertain whether *T. aquaticus* and *T. thermophilus* are capable of chemolithoautotrophic growth by arsenite oxidation. Using low levels (0.020 and 0.002% yeast extract; w/v) and the absence of a carbon source, cultures were incubated with and without arsenite present. Growth in these experiments was extremely slow, and cultures grown with arsenite showed no change in their growth rate compared to cultures grown in the absence of arsenite (data not shown).

Field Investigations. *Physical and Geochemical Parameters.* The Twin Butte Vista Hot Spring is comprised of a small pool with a vent at the western edge and overflow waters draining in three channels (Figure 3). The flow rate in the two western channels was irregular, increasing with sporadic surges from the vent, and samples from these drainages were not used in this study. Waters overflowed via the northern channel at a nearly constant rate, buffered by the deep pool between the vent and outlet. The north drainage
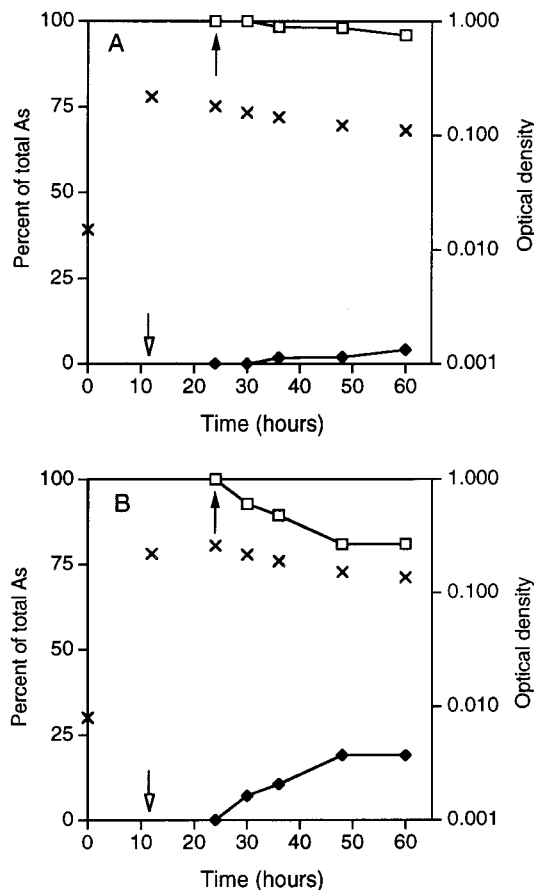

FIGURE 2. Arsenic speciation and culture density during antibiotic-inhibited culturing of *Thermus aquaticus* YT1 (A) and *Thermus thermophilus* HB8 (B). Open arrows indicate the addition of 2.0 mg L^{-1} of kanamycin and 2.0 mg L^{-1} of ampicillin. Filled arrows indicate the addition of 75 mg L^{-1} of As(III). Linear plot: □, arsenite; ◆, arsenate. Logarithmic plot: ×, optical density of culture.
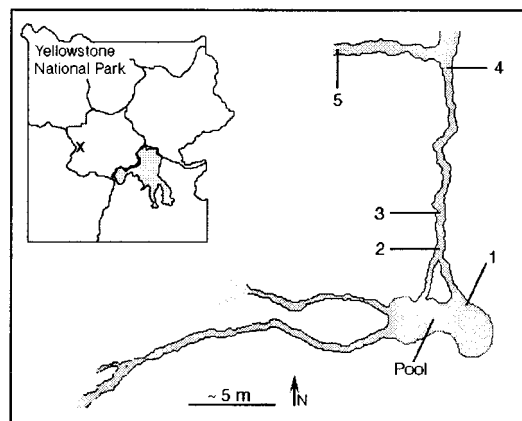

FIGURE 3. Schematic map of the Twin Butte Vista Hot Spring, its drainages, and sampling locations.

channel was very well confined and the residence time for waters in the sampled region (flow from station 1 to 5) was estimated to be approximately 2 min.

Geothermal waters venting the Twin Butte Vista Hot Spring were alkaline, with an average pH of 8.8 throughout the north drainage channel (Table 1). Water temperatures decreased from 82.6 to 65.1 °C during flow from station 1 to 5. Conditions were reducing at the pool, becoming more oxidizing with distance as Eh increased from −87.2 mV at station 1 to 3.3 mV at station 5. Sulfide concentrations

TABLE 1. Physical and Chemical Characteristics of the Twin Butte Vista Hot Spring North Drainage Channel

station number	1	2	3	4	5	field blank[b]
ca. distance to pool (m)	0	3.8	6	13.5	18.5	
temp (°C)	82.6	79.0	77.7	72.6	65.1	
pH	8.7	8.7	8.7	8.8	8.9	
Eh (mV)	−87.2	−76.6	−36.8	−62.1	3.3	
conductance (μS/cm)	1449	1460	1471	1479	1497	
Al[a]	0.26	0.28	0.3	0.33	0.33	<0.08
B	3.2	3.2	3.2	3.1	3.2	<0.003
Ba	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Be	<0.0001	0.0004	0.001	0.002	0.002	<0.0001
Ca	0.22	0.24	0.35	0.46	0.46	<0.05
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	<0.002	<0.002	<0.002	0.002	0.002	<0.002
Cu	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
K	12	12	12	13	13	0.012
Li	3.2	3.5	3.5	3.6	3.7	<0.008
Mg	<0.0001	<0.0001	<0.0001	0.001	0.001	<0.06
Mn	<0.001	<0.001	<0.001	0.002	0.002	<0.001
Na	320	310	310	330	340	<0.04
Ni	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Pb	<0.006	<0.006	<0.006	<0.006	0.006	<0.006
SiO$_2$	190	270	240	170	190	<0.01
Se	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Sr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn	<0.001	<0.001	<0.001	0.002	0.001	<0.001
Fe(total)	0.046	0.016	0.015	0.014	0.002	0.004
Fe(II)	0.021	0.009	0.006	0.002	<0.002	
F	21	22	21	21	22	<0.05
Cl	280	280	280	280	280	<0.4
Br	0.99	0.86	0.94	0.97	0.96	<0.1
NO$_3$	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
SO$_4$	16	16	16	16	17	<0.8
S^{2-}	0.13	0.13	0.10	0.04	0.017	
alkalinity (as HCO$_3$)	337	341	345	347	355	

[a] Concentrations are in mg L^{-1}. [b] "Field blank" was deionized water prepared in the field (filtered and acidified) and analyzed under the same conditions as the hot spring samples.

TABLE 2. Arsenic Measurements for the North Drainage Channel of the Twin Butte Vista Hot Spring

	As (total), mg L^{-1}			As(III), mg L^{-1}			As(V), mg L^{-1} by difference
sample	n^a	mean	±	n^a	mean	±	
station 1	2	2.5	0.01	3	1.9	0.04	0.6
station 2	2	2.5	0.02	3	1.7	0.05	0.8
station 3	2	2.5	0.02	3	1.3	0.02	1.2
station 4	2	2.5	0.00	3	1.0	0.04	1.5
station 5	2	2.5	0.02	3	0.61	0.03	1.9
field blank[b]	3	<0.0001		2	<0.0002		

[a] Replicate analyses (n) were performed for each sample. Error (±) is equal to the range (for $n = 2$) or relative standard deviation (for $n = 3$). [b] "Field blank" was deionized water prepared in the field (filtered and acidified) and analyzed under the same conditions as the hot spring samples.

decreased from 0.13 to 0.017 mg L^{-1} as sulfate remained nearly constant between the first and final sampling stations. Total and reduced iron species were very low in the drainage fluids (0.046 mg L^{-1} total Fe at station 1) and decreased with distance. Results of additional chemical analyses are shown in Table 1.

The total dissolved arsenic concentration was consistent at 2.5 mg L^{-1} throughout the north drainage channel (Table 2). While total As behaved conservatively, speciation changed dramatically as waters flowed downstream. As(III) was dominant at the first sampling station at 1.9 mg L^{-1} and decreased at each subsequent station to 0.61 mg L^{-1} at the final point. Correspondingly, As(V) was low near the pool at 0.6 mg L^{-1} and increased with distance to 1.9 mg L^{-1} at the final sampling station. The rate of arsenite oxidation between stations 1 and 5 was estimated to be approximately 0.5 mg L^{-1} min^{-1}.

Laboratory experiments were performed to test for catalysis of As(III) oxidation by mineral surfaces. Sediments collected from station 1 (150 mg) were autoclaved and placed in a flask with 10 mL of filter-sterilized fluids collected from the same location. A spike of 75 mg L^{-1} of As(III) was added to the flask which was then incubated for 48 h at 70 °C with 125 rpm shaking. The rate of arsenite oxidation in this experiment was linear at 0.006 mg L^{-1} min^{-1}. At this rate, 0.012 mg L^{-1} of As(III) in the Twin Butte Vista Hot Spring drainage would have been oxidized abiotically during flow from station 1 to station 5.

Microbial Characterizations. Pale-orange biofilms were visible beginning ~2 m downstream of station 1 and were evident in the remainder of the channel. These microbial streamers, attached to sediments and other surfaces, were often very dense and formed thick filaments up to 10 cm long (Figure 4). Microscopic observations revealed a very low cell density at station 1 consisting primarily of cocci. Stations 2 and 3 were dominated by dense, homogeneous masses of thin filamentous rods. Samples from stations 4 and 5 also contained large accumulations of thin filamentous rods in addition to clusters of thick green rods (likely cyanobacteria).

To label individual cells and quantify their relative proportion of the microbial community at each station, fluorescence in-situ hybridizations were performed. Results of FISH analyses are shown in Figure 5. The probes Arch915 and Eub338, specific for the archaeal and bacterial domains

FIGURE 4. Microbial biofilms in the north drainage channel near station 2 of the Twin Butte Vista Hot Spring. Sample tube in upper-right is 3 cm in diameter.
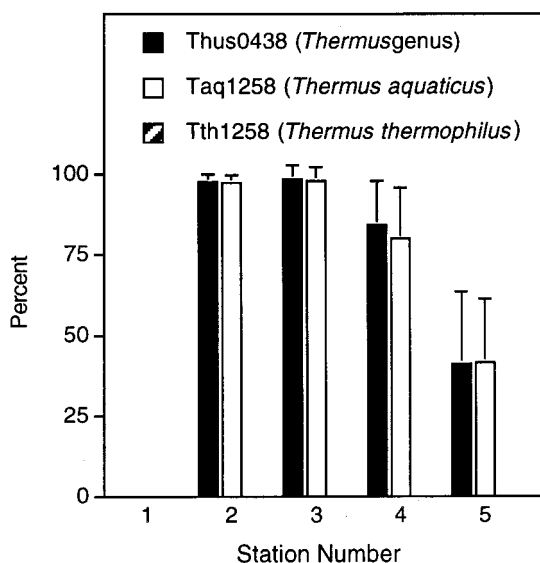

FIGURE 5. Quantification of the genus *Thermus* and the species *Thermus aquaticus* as determined by FISH. *Thermus thermophilus* was not detected. Values are expressed as the percent of (Arch915 + Eub338) hybridized cells. Error bars represent 1 SD; only the positive portions of error bars are shown.

respectively, were used to label all viable prokaryotic cells. Thus 0438, specific at the genus level, was used to detect *Thermus* species. The species-specific probes Taq1258 and Tth1258 were used to identify and enumerate *Thermus aquaticus* and *Thermus thermophilus*, respectively.

At station 1, no cells were detected with the genus- or species-specific probes, indicating that *Thermus* species were not present in the pool. As the hot spring waters cooled with distance, *Thermus aquaticus* was found to be colonizing the drainage channel at stations 2 and 3, occurring as nearly 100% of the microbial population. Stations 4 and 5 contained

a lower percentage of *Thermus aquaticus* (80 and 42%, respectively), although the species remained prominent. *Thermus thermophilus* was not detected in any of the drainage samples.

Discussion

Laboratory culturing experiments demonstrated rapid arsenite oxidation by *Thermus aquaticus* and *Thermus thermophilus*. Biological rates of oxidation were more than 100-fold greater than the abiotic oxidation rate. Decreased rates of arsenite oxidation by antibiotic-treated cultures indicate that active and growing cells are required for the oxidation process. *T. aquaticus* and *T. thermophilus* were not able to grow with arsenite as a sole energy source and catabolic energy was not derived from arsenite oxidation. Considering that arsenate is less toxic than arsenite (*38*), the ecological role of arsenite oxidation by these organisms is likely detoxification. This study of *T. aquaticus* and *T. thermophilus* presents the first report of arsenic oxidation by a *Thermus* species. The occurrence of arsenite oxidation among other *Thermus* species awaits further investigation.

To examine the impact of *T. aquaticus* and *T. thermophilus* on arsenic speciation in the environment, a field study was conducted at Yellowstone National Park. Geothermal fluids at YNP often contain high levels of total dissolved inorganic arsenic (up to 3 mg L^{-1}) (*39*) and *Thermus* species have been found in abundance near many geothermal features (*24*). The Twin Butte Vista Hot Spring in particular was reported to contain dense biofilms of cells identified as a *Thermus* species based on morphologic observations (*24*).

Investigations at the Twin Butte Vista Hot Spring indicate that rapid arsenite oxidation occurs as geothermal waters flow through the drainage channel. The rate of arsenite oxidation by sterilized sediments and fluids was approximately equal to the rate observed in abiotic culturing experiments and significantly slower than the rate measured in the field. These results strongly suggest a biological catalyst

is involved in the arsenite oxidation process in this environment.

The drainage channel of the Twin Butte Vista Hot Spring was densely colonized by *Thermus aquaticus*. Variables such as cell growth rate and total cell numbers do not allow for a direct comparison of biological As(III) oxidation rates in the laboratory and in the field. However, pure culture experiments clearly indicated that *T. aquaticus* has the capacity to function as a catalyst of arsenite oxidation. The combined field and laboratory results presented here provide strong evidence that *Thermus aquaticus* colonizing arsenic-rich geothermal environments accelerates As(III) oxidation and can significantly impact As speciation in nature.

Previous studies have shown that the extent of arsenic adsorption to iron oxyhydroxides and many other mineral surfaces changes with arsenic speciation (*40*). Arsenate is generally more readily adsorbed than arsenite and therefore considered less mobile. However, geothermal fluids within the Twin Butte Vista Hot Spring drainage channel are characterized by high temperature, high pH, and low iron concentrations—conditions which favor conservative arsenic transport (*20*). As a result, a loss of total dissolved arsenic was not observed as arsenite was quickly oxidized to arsenate. Nonetheless, decreased As(III) to As(V) ratios resulting from rapid microbial arsenic oxidation at the source of geothermal arsenic input to the environment will likely enhance adsorption to soil and sediments as these fluids flow downstream and mix with lower temperature iron-bearing waters. Acceleration of the conversion of arsenite to arsenate by *Thermus aquaticus* and *Thermus thermophilus* may therefore be critical in controlling arsenic bioavailability and toxicity in the many thermal environments where these organisms are known to occur.

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