

Thermophilic Magnetotactic Bacteria from Mickey Hot Springs, an Arsenic-Rich Hydrothermal System in Oregon

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ABSTRACT: Magnetotactic bacteria (MTB) thrive in aquatic sediments all over the world, but their complete role(s) in their geobiological habit as well as their significance in the fossil record remains unresolved. We were able to collect, enrich, and purify MTB from Mickey Hot Springs, a unique arsenic-rich hot spring system located in the Alvord Desert of southeastern Oregon. Populations of MTB were present in water and surface sediment samples in a geothermal spring at temperature 47 °C, pH of 8.0, and 0.96 mg/L arsenic. Using 16S rDNA analysis, the organism was found to belong to the phylum *Nitrospirae*. Cells had rod to vibrioid morphology, a single flagellum and single magnetosome chain as determined by scanning transmission electron microscopy (STEM). The magnetosomes contained bullet-shaped crystals of magnetite (Fe₃O₄) approximately 84 nm long and 39 nm wide. This is the first instance of MTB collected from an arsenic-rich, moderately thermophilic



environment. The discovery of MTB at this site extends the limits of habitats for these bacteria and provides a proxy for the search for magnetofossils in the rock record.

KEYWORDS: magnetotactic, biomineralization, magnetite, Mickey Hot Springs, arsenic, thermophilic, hydrothermal

1. INTRODUCTION

Magnetotactic bacteria (MTB) are found in a variety of aquatic sediments such as marine and freshwater environments all over the world.¹⁻⁹ MTB play an important part in the geochemical cycling of elements such as carbon, iron, nitrogen, phosphorus, and sulfur.¹⁰⁻¹⁷ They are known to occupy specific niches (e.g., low O_2 concentrations) within their habitat using chemotaxis and magnetotaxis to find optimal growth conditions.^{1,16,18–20} Different chemicals in the environment such as iron, nitrate, oxygen, and sulfur can impact the type of MTB that exist at a specific environmental site.^{13,21-24} Previous studies have described the metabolic diversity of MTB within various habitats and confirm that these organisms play important biogeochemical roles in their environment.^{7,13,19,21,25,26} For example, Araujo et al. (2016) used a comprehensive genomic approach to characterize Magnetofaba australis strain IT-1 and demonstrate that it is capable of nitrogen fixation, sulfur oxidation, and reduction and synthesis of intracellular phosphate-rich granules.²¹ In another paper, Rivas-Lamelo et al. (2017) demonstrated that MTB belonging to the Magnetococcaceae family play an important role in the phosphorus cycle in Lake Pavin (France).

The unique feature common to all MTB is their ability to synthesize intracellular membrane-bounded crystals of single domain magnetite (Fe₃O₄), greigite (Fe₃S₄), or both.^{2,6,27–29}

MTB are commonly found in chemically stratified water or sediment at the oxic–anoxic interface. The magnetosomes (i.e., membrane bound magnetic crystals) are arranged in a chain within the bacteria and provide a torque on the cells that passively aligns them with Earth's geomagnetic field. One hypothesis is that this alignment in turn reduces a bacterium's navigational route from three-dimensions to one-dimension, thus shortening the time and decreasing the amount of energy it takes for cells to navigate to their preferred habitat, the oxic– anoxic interface near the bottom of bodies of water.^{2,30–32}

MTB do not fall within one phylogenetic group, but rather are polyphyletic and have been found in several phyla including *Alphaproteobacteia*, *Etaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Latescibacteria*, *Nitrospirae*, *Omnitrophica*, and *Planctomycetes*.^{1,9,33–44} MTB are common in freshwater and marine environments, and studying MTB from environmental sites has progressed in recent years due to new biochemical and molecular based approaches and advances in

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instrumentation and techniques. These advancements have allowed researchers to study magnetotaxis and biomineralization in uncultivated MTB.^{7,9,34,37,42,45–49} Studies have also investigated MTB living in different habitats and found that the MTB community can change over time as environmental conditions change.^{48,50–52}

Magnetite crystals isolated from MTB have a very specific size range, a well-defined and consistent morphology, and are relatively chemically pure (exclusively Fe_3O_4).^{53,54} Such minerals are preserved in the rock record as magnetofossils, which have been found in Mesozoic rocks, and may extend back as far as the Precambrian.^{55–58} Differentiating between magnetite made by MTB and abiogenic magnetite in the fossil record is not straightforward, but there are distinctions between the two, such as size, uniformity, high chemical purity, and sometimes the magnetosome chain is preserved.^{53,55,57,59–67}

Recently MTB have been found living in more extreme environments like the geothermal springs of the Great Basin region of the United States, which includes parts of California, Nevada, and Oregon.^{5,67–69} A moderately thermophilic MTB, having an upper growth limit of around 63 °C, was isolated from Great Boiling Springs in northern Nevada.⁵ This same group isolated obligately alkaliphilic MTB from Mono Lake, California, which could only grow from pH 8.0–9.5.⁶⁷ Mickey Hot Springs is a geothermal system located in southeastern Oregon (USA) and, like Great Boiling Springs and Mono Lake, is part of the Great Basin. Of the three sites, Mono Lake (CA) is the southernmost geothermal spring. Great Boiling Springs (NV) sits approximately 325 km north of Mono Lake, and Mickey Hot Springs (OR) is located approximately 300 km northeast of Great Boiling Springs (NV).

Mickey Hot Springs consists of numerous geothermal springs characterized by temperatures ranging from 25 to 100 °C, and water pH ranging from neutral to slightly alkaline. Unlike other hot springs in the Great Basin, Mickey Hot Springs also contains high concentrations of arsenic (1 mg/L), a heavy metal that is toxic to many organisms. The unique geochemistry of Mickey Hot Springs has been described extensively in various studies. $^{70-73}$ Despite its extreme conditions, Mickey Hot Springs is known to contain diverse bacterial communities and fossilized organisms that live, or have lived, in these springs.74-77 Although no MTB had previously been identified at Mickey Hot Springs, we believed that MTB could live in its extreme environment because of the following reasons: (1) Mono Lake, Great Boiling Springs, and Mickey Hot Springs are all located within the Great Basin region of the USA; (2) Mickey Hot Springs has similar conditions (i.e., temperature and pH) to Great Boiling Springs and Mono Lake; (3) magnetotactic bacteria have previously been isolated from Great Boiling Springs and Mono Lake; (4) nonmagnetotactic have previously been isolated from Mickey Hot Springs.

In this study, we investigated whether MTB exist in the extreme arsenic-rich thermal spring system at Mickey Hot Springs. It would be the first time that MTB have been found living in an arsenic-rich (1 mg/L) and moderately thermophilic environment. Such a discovery would reveal a greater diversity of MTB than is currently known. By investigating different geographical locations and unusual habitats (e.g., high arsenic, high temperature, high pH) we hope to expand our understanding of these microorganisms and determine when and why magnetotaxis first evolved on Earth. Furthermore,

discovering new MTB from unique habitats could potentially reveal different types of metabolism, or yet unrecognized functions for magnetosomes.

This investigation at Mickey Hot Springs has uncovered MTB that belong to the phylum Nitrospirae. This is the first time that MTB have been found living in an arsenic-rich and moderately thermophilic environment. Transmission electron microscopy (TEM) was used to determine the morphology of the cells, size and shape of magnetosomes, while scanning transmission electron microscopy (STEM) coupled with energy-dispersive spectroscopy (EDX) was used to determine the chemical composition of the magnetosomes contained within the MTB. This discovery will likely be of interest to scientists searching for the biosignatures of life on Earth, as Mickey Hot Springs is known to contain microbial fossils in sinter.⁷⁶⁻⁷⁸ In addition, this work will likely be of profound interest to those searching for evidence of life on other planets where similar environmental conditions may be common (e.g., hot spring sinters on Mars).⁷

2. EXPERIMENTAL METHODS

Sample Collection. Sediment and water were obtained at Mickey Hot Springs (42°40′42.6″N, 118°20′54.2″W) by collecting the sediment approximately 20-45 cm below the surface of the water using sterile, plastic 1-L containers. Each container (2 total) contained one-half to three-quarters sediment and the remainder was filled with water from the site. The bottles were sealed and brought back to the lab for analysis. No unexpected or unusually high safety hazards were encountered. Samples were kept in the dark at elevated temperature (40–45 $^{\circ}$ C). A portion of the sample (~500 mL) was kept at room temperature (~ 22 °C). There did not appear to be a significant difference in the number of magnetotactic cells between the two temperatures, suggesting that the MTB isolated from Mickey Hot Springs could tolerate temperatures down to at least 22 °C. Using microscopy, we estimated their numbers to be between 10^2 to 10^4 cells mL⁻¹. Samples kept at elevated temperature were used for MTB enrichment, electron microscopy, and phylogenetic analysis.

At the time of sediment collection, a water sample was obtained along with measurements of the water temperature and pH at the collection site. Samples of sediment were placed into sterile plastic containers and transported back to the laboratory for trace element analysis. Sediment pore water samples were extracted by centrifugation at 5000 rpm for 15 min, passed through a 0.22 μ m filter, acidified in 5% nitric acid (v/v), and sealed. The water samples were analyzed at The Ohio State University Trace Element Research Laboratory using a PerkinElmer Optima 3000DV inductively coupled plasma optical emission spectrometer. Samples were analyzed for boron (B), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), arsenic (As), aluminum (Al), copper (Cu), phosphorus (P), iron (Fe), and silicon (Si).

Magnetotactic Bacteria Enrichment. MTB were collected and purified from sediment and water samples.⁷⁹ Briefly, the south end of a magnet was placed against the exterior of the container just above the sediment-water interface. At the opposite side of the container, this was repeated for the north end of another magnet. After 1 h the water around the south end of the magnet was extracted with a pipet and placed in a racetrack with a cotton plug at the sealed end.^{80,81} This was repeated 12 times for each sample. A magnet was placed at the sealed end, and the magnetotactic cells were allowed to swim



Figure 1. Mickey Hot Springs, Oregon (USA) field site $(42^{\circ}40'42.6''N, 118^{\circ}20'54.2''W)$. (A) Photograph showing view of upper main pool (called Morning Glory) with a narrow channel of water flowing, by gravity, out of the upper pool into a smaller lower pool (white dotted arrow). (B) Photograph of lower pool where sediment and water samples were collected. The black arrow points to the 1 L container used to collect the sample from the lower pool. At the time of collection, the lower pool had a temperature of 47 °C and pH of 8.0. (C) Transmission electron microscope (TEM) image of a magnetotactic bacterium (MTB) collected and purified from the sediment and water sample shown in (B). Cells were rod to vibrioid shaped with a single polar flagellum (black arrow). The magnetosome chain contained bullet-shaped crystals (white arrow). Scale bar is 500 nm.

through the cotton barrier for approximately 30 min. The racetrack was taken away from the magnet, the tip snapped off, and the bacteria removed with a syringe. A total of 500–1000 μ L were collected from each sample. The hanging drop method was used to confirm the presence of MTB in the sample.^{79,82}

Electron Microscopy and Energy Dispersive X-ray Spectroscopy. An aliquot of the enriched cells was placed on a 200-mesh copper grid coated with carbon and Formvar (Ted Pella), washed one time by placing the grid on a drop of water for 30 s, and then dried using a piece of filter paper. The grid was analyzed in an FEI Tecnai G² Spirit transmission electron microscope (TEM) or an FEI Tecnai F20 scanning transmission electron microscope (STEM) using high angle annular dark field (HAADF) mode.^{68,69,77,79,83} The accelerating voltage of the G² Spirit was 80 keV, spot size 2, and using the number 2 objective aperture, and images were collected using a Gatan camera and AMT Image Capture software. For the Tecnai F20, an accelerating voltage of 200 keV was used in the HAADF mode. Magnetosome crystals inside the cells were analyzed using the Energy Dispersive X-ray (EDX) spectrometer on the F20, only a 100 μ m condenser aperture was used, and the specimen was tilted 5° toward the detector which was an EDAX detector with an ultrathin Moxtek AP3.3 window with an elevation angle of 20° . The size of the cells and the magnetite crystals were analyzed using FIJI software (https://cemas.osu.edu/capabilities).

Phylogenetic Analysis. Approximately 500 μ L of sample collected from the racetrack was used to obtain DNA for phylogenetic analysis. DNA was obtained from the cells by homogenizing the cells and resuspending them in RLT buffer (Qiagen) with β -mercaptoethanol. A Qiagen DNA kit was used to isolate the DNA. Fragments of the DNA were amplified using PCR using 28F and 519R primers (TTTGA-

TCNTGGCTCAG, GWNTTACNGCGGCKGCTG) and a Qiagen Hotstar Taq Master Mix. The DNA was denatured at 95 °C for 5 min, then 35 cycles at 94 °C for 30 s, 54 °C for 45 s, 72 °C for 60 s, and final extension at 72 °C for 10 min. PCR amplification, library preparation and 16S rRNA gene sequence analysis were done at RTL Genomics (Lubbock, Texas). RTL Genomics also performed quality checking, OTU selection, FASTA formatted sequences, sequence clustering, tree building, and taxonomic identification.

Evolutionary relationships were inferred using the UPGMA method.⁸⁴ The phylogenetic tree shown in the Results section is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were computed using the Maximum Composite Likelihood Method⁸⁵ and are in the units of the number of base substitutions per site. The analysis involved 24 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1793 positions in the final data set. Evolutionary analyses were conducted in MEGA5.⁸⁶

3. RESULTS

Sample Collection. Sediment and water samples were collected from a geothermal spring located within Mickey Hot Springs (Figure 1A and 1B) in southeastern Oregon (USA). This site was selected because it represented a unique habitat (e.g., high arsenic, moderately thermophilic) where MTB had never been found and could potentially harbor a diverse population of novel prokaryotes.

Phylogenetic Analysis. 16S rRNA gene sequence analysis was conducted on enriched MTB cells. An aliquot containing enriched MTB from Mickey Hot Springs was analyzed by 16S sequencing from all the bacteria contained within the enriched sample. There were a total of 29 different 16S rRNA gene



Figure 2. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences. Phylogenetic position of samples collected from Mickey Hot Springs (MHS-1, shown in bold) belong to the phylum *Nitrospirae*. GenBank accession numbers are provided in parentheses. A *Magnetospirillum* species, an alphaproteobacterial MTB (GenBank accession number GU271691) was used as a reference point to root the tree. Scale bar represents 5% sequence divergence.

sequences and 15 sequences were grouped together as MHS-1 (Figure 2). These sequences were compared with sequences in the NCBI nucleotide database to determine how similar they are to other sequences. On the basis of the percentage of similarities, a phylogenetic tree was constructed in MEGA5⁸⁶ to show how the sequences compared to other MTB. The 16S rRNA gene analysis placed the organisms purified from Mickey Hot Springs within the phylum *Nitrospirae* (Figure 2). Two other magnetotactic bacteria, *Magnetobacterium bavaricum*⁸⁷ and an uncultured *Magnetobacterium* sp.,⁸⁸ both belonging to the phylum *Nitrospirae*, were found to be the closest relatives to MHS-1 (Figure 2).

STEM and EDX Analysis. STEM and EDX were used to examine the morphology of the enriched MTB cells, their magnetosomes, as well as the composition of the magnetosome crystals. The sample contained one type of cell morphology that was rod to vibrioid shape (Figure 1C). The average length and width of each bacterium was 2.2 (\pm 0.6) μ m and 0.62 (\pm 0.1) μ m, respectively (n = 37). Each cell had a single polar flagellum (Figure 1C). The magnetosome chain was usually single, but sometimes two chains would partially overlap. In three cells, two distinct magnetosome chains were identified. Each chain averaged 16 (\pm 5) magnetosomes per chain (n = 50).

EDX analysis established that the magnetosome crystals were composed of iron and oxygen (Figure 3A and 3B). The crystals were bullet-shaped and typically aligned with the sharp end of one crystal next to the flat end of the next crystal; however, some crystals were aligned with their flat ends against each other (Figure 3A). The crystals had an average length of 84 (±17) nm (range 40 to 136 nm) and an average width of 39 (±8) nm (range 17 to 63 nm) (n = 377) (Figure 4). A plot (Figure 4D) of crystal length versus shape factor (width/length) shows that the crystals fit the theoretical single-magnetic-domain size range.⁸⁹

4. DISCUSSION

Here we report the discovery of an interesting MTB present at Mickey Hot Springs (Oregon, USA). This is a fascinating geothermal spring system because it has moderately high temperature (45–50 °C), high arsenic concentration (\sim 1 mg/L), and is conducive to the formation of microfossils in sinter deposits. To our knowledge, this is the first time that MTB have been found living in an arsenic-rich and moderately thermophilic environment.

Mickey Hot Springs is located at the northern end of the Alvord Basin in southeastern Oregon at an elevation of approximately 1200 m. This area possesses several active hot springs generated from fluids that ascend through deep faults which discharge at the surface.⁹⁰ Consequently, the fluid contains dissolved ions, one of which is arsenic (Table 1).^{73,90,91} Mickey Hot Springs has a very high concentration of arsenic, almost 1 mg/L (Table 1). For reference, in 2001 the U.S. Environmental Protection Agency (EPA) set the standard for arsenic in drinking water of 0.01 mg/L.⁹² Water samples from Mickey Hot Springs also contain high concentrations of silicon and sodium 76 mg/L and 470 mg/L, respectively (Table 1). The spring where the samples were collected had a temperature of 47 °C and the pH was 8.0 (Figure 1B, Table 1).

The MTB discovered at Mickey Hot Springs synthesize elongated, bullet-shaped magnetite crystals, which are thought to be the most ancient type of crystal biomineralized by MTB.^{39,93,94} This is notable because elongated, bullet-shaped magnetite crystals could serve as an ideal biomarker at Mickey Hot Springs as such crystals are not known to form inorganically.^{38,68,69} If magnetofossils could be found in sinter deposits at Mickey Hot Springs, they might serve as a unique biosignature for ancient lifeforms and facilitate the characterization of the paleoenvironment at the site.

MTB from Mickey Hot Springs were rod to vibrio-shaped and were 2.2 μ m long by 0.6 μ m wide (Figure 1). Most of the cells contained a single chain of magnetosomes (Figures 1C and 3A). Each magnetosome had approximately 16 bulletshaped crystals that were, on average, 84 nm long by 39 nm wide (Figure 4). All 15 operational taxonomic units (OTUs) obtained from the 16S rRNA gene sequence analysis and grouped as MHS-1 belonged to the *Nitrospirae* phylum (Figure 2). The association between their phylogenetic position (i.e., phylum) and shape of their magnetosomes is consistent with other studies, as all known *Nitrospirae* MTB contain bulletshaped magnetite crystals^{5,38,68,69,95} like the ones described herein for Mickey Hot Springs.

MTB have previously been found at Great Boiling Springs (Nevada),^{5,96} which like Mickey Hot Springs, is located within the United States' Great Basin. Mickey Hot Springs, Oregon is approximately 300 km northeast of Great Boiling Springs, Nevada. The geochemistry of Great Boiling Springs has been described previously.^{5,97,98} The main differences between the two sites are (i) Mickey Hot Springs contains high concentrations of arsenic (~1 mg/L) and (ii) the water temperature measured at Mickey Hot Springs (47 °C) was 16 °C lower than the water temperature measured at Great Boiling Springs (63 °C) (Table 2).

MTB isolated from Great Boiling Springs (named HSMV-1) were able to survive in the laboratory for several months at temperatures ranging from 25 to 63 °C.⁵ The upper limit of this temperature range was higher than the temperature measured at Mickey Hot Springs (47 °C, Tables 1 and 2).



Figure 3. STEM-EDX analysis of MTB collected from Mickey Hot Springs. A single magnetotactic bacterium with bullet-shaped crystals aligned in a single chain (A). The two inset panels show where EDX spectra were collected for a crystal (red circle) and for the cell background (red rectangle). Scale bars are 500 nm. (B) EDX spectrum for the bullet-shaped crystal (red circle) showed iron (Fe) and oxygen (O) as its major components, and copper (Cu) from the STEM support grid. (C) EDX spectrum for the cell background (red rectangle) showed peaks for Fe, O, and Cu (from the STEM support grid) as well as arsenic (As). Also shown are peaks for silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K).

HSMV-1 cells contained, on average, 12 ± 6 bullet-shaped magnetite magnetosome crystals per cell that were 113 ± 34 nm by 40 ± 5 nm in size (Table 2).⁵ HSMV-1 cells were approximately 2 μ m by 0.5 μ m, had a single polar flagellum and each cell contained a single chain of magnetosomes (Table 2).⁵

MTB purified from Mickey Hot Springs had similar, but not identical, characteristics to HSMV-1 purified from Great Boiling Springs (Table 2). MTB from both sites were similar in that both bacteria were phylogenetically grouped within the phylum *Nitrospirae* and both bacteria synthesized a single magnetosome chain that contained bullet-shaped magnetite crystals (Table 2). Crystals from both sites were found to fit (i.e., shape factor) within the theoretical single-domain size range and in agreement with all known mature magnetosome Fe_3O_4 crystals (Table 2).^{88,89} Single domain magnetite crystals are the ideal size for MTB because they have the maximum possible magnetic moment per unit volume.^{1,2,30} Aligning these crystals in a linear magnetosome chain optimizes magnetotaxis.^{1,2,30} Smaller crystals are superparamagnetic and can flip directions, while larger crystals can form multiple



Figure 4. Characterization of magnetosomes contained within MTB from Mickey Hot Springs. (A) Frequency of the shape factor demonstrating that the crystal length is approximately twice as long as width. (B) Length versus width for magnetosomes (black dots). The black dashed line represents width equal to length. (C) Frequency versus width of crystals demonstrates narrow size distribution of magnetie. (D) The sizes of magnetic nanocrystals from Mickey Hot Springs (black dots) fall within the single domain size range based on Butler and Banerjee (1975).⁸⁹

Table 1.	Chemistry of M	Aickey H	lot Springs V	Where 1	Magnetotactic	Bacteri	a Were C	ollected ^a	
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pН	As (mg/L)	Al (mg/L)	B (mg/L)	Ca (mg/L)	Cu (mg/L)
8.0 ± 0.2	0.96 ± 0.11	0.13 ± 0.001	8.2 ± 0.98	0.30 ± 0.05	0.005 ± 0.001
Fe (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	P(mg/L)	Si (mg/L)
0.03 ± 0.002	0.19 ± 0.01	470.0 ± 22.5	41.0 ± 1.8	0.06 ± 0.01	76.0 ± 3.63

^aShown are the averages ± standard errors of duplicate measurements. Temperature of geothermal pool at time of sampling was 47 ± 1.6 °C.

domains that work against each other and oppose the desired direction. 1,2,30,89

MTB from Mickey Hot Springs had, on average, more magnetite crystals per cell than HSMV-1. HSMV-1 from Great Boiling Springs had 12 \pm 6 crystals per cell,⁵ whereas MTB from Mickey Hot Springs had 16 \pm 5 crystals per cell (Table 2). HSMV-1 produced magnetosome crystals that were slightly longer (i.e., 35%) but equal in width to those synthesized by MTB from Mickey Hot Springs. MTB from both sites have a similar cell size (i.e., HSMV-1 cells were 1.8 μ m × 0.4 μ m; Mickey Hot Springs cells were 2.2 μ m × 0.6 μ m), both have similar cell morphology (i.e., vibrioid shape) and both use a single polar flagellum for motility (Table 2).

The most notable differences between the two sites (i.e., Great Boiling Springs and Mickey Hot Springs), was the high concentrations of arsenic ($\sim 1 \text{ mg/L}$) measured at Mickey Hot Springs. This is significant because arsenic is considered universally toxic to organisms.^{99–101} Yet, MTB were found growing in Mickey Hot Springs where arsenic levels were as high as $\sim 1 \text{ mg/L}$. Using a microscope, we estimated the

number of MTB to be between 10^2 to 10^4 cells mL⁻¹ in sample bottles collected at Mickey Hot Springs. EDX data collected on bacterial magnetosomes from Mickey Hot Springs showed no arsenic within the magnetite crystals (Figure 3B). Arsenic was only detected in the bacterial cell (Figure 3C). The high-arsenic environment of Mickey Hot Springs apparently does not affect the synthesis of magnetite within the bacteria (Figure 3).

Magnetite has been shown to remove arsenic from solution.¹⁰² Recently, genetically modified MTB have been engineered to act as whole-cell biosensors for the detection of arsenic in drinking water.¹⁰³ Therefore, the ability of MTB to tolerate high arsenic concentrations may be useful to humans because MTB have the capacity to be used in remediating arsenic contaminated sites or could be utilized as metalloid biosensors for public health programs.^{102,103}

Environmental parameters have been shown to affect the mineral crystals contained within magnetosomes.^{54,104} The chemical, physical, and magnetic properties of magnetosomes from both cultured and uncultured MTB can be impacted by

Table 2. Comparison of MTB from Mickey Hot Springs in Oregon (USA) to MTB from Great Boiling Springs in Nevada (USA)^a

Geothermal Spring	MTB from MHS	MTB from GBS (HSMV- 1)
Name of spring	Mickey Hot Spring (MHS)	Great Boiling Springs (GBS)
Location of spring	Princeton, Oregon, USA	Gerlach, Nevada, USA
Temperature of spring	45 to 49 °C	32 to 63 °C
Coordinates	42°40′42.6″N, 118°20′54.2″W	40°39′41.8″N, 119°21′59.8″W
pH of spring	7.8 to 8.2	6.4 to 7.5
MTB cell	Gram-negative, Vibrioid	Gram-negative, Vibrioid
MTB cell length	$2.2 \pm 0.6 \ \mu \mathrm{m}$	$1.8 \pm 0.4 \ \mu m$
MTB cell width	$0.62 \pm 0.1 \ \mu m$	$0.4 \pm 0.1 \ \mu m$
Phylum	Nitrospirae	Nitrospirae
Number of flagella	1 polar flagellum	1 polar flagellum
Magnetosome chain	Single chain along long axis	Single chain along long axis
Magnetosome shape	Bullet	Bullet
Magnetosome per cell	16 ± 5	12 ± 6
Magnetosome length	84 ± 17 nm	113 ± 34 nm
Magnetosome width	39 ± 8 nm	40 ± 5 nm
Mineral type	Magnetite	Magnetite
Magnetite grain size	Single domain size range	Single domain size range

⁴⁷The magnetotactic bacteria from Great Boiling Springs is known as strain HSMV-1. Data for HSMV-1 obtained from Lefèvre et al. (2010).⁵ Theoretical single-domain size range for magnetic nanocrystals determined from Butler and Banerjee (1975).⁸⁹ GBS, Great Boiling Springs; MHS, Mickey Hot Springs.

the chemical composition of the growth medium.^{105,106} The unique geochemistry at Mickey Hot Springs does not seem to affect the chemical composition or crystal morphology of the magnetite crystals contained within the magnetosomes (Figures 1, 3, and 4). On the basis of the magnetosome crystal morphology and 16S rRNA gene analysis, the unique habitat found at Mickey Hot Springs does seem to select for one type of MTB (Figure 2), those that are affiliated with the phylum *Nitrospirae*. No MTB in the class of *Alphaproteobacteria*, common in many sedimentary and aquatic environments, were found at Mickey Hot Springs (Figure 2).

It is possible that there was a selection bias for *Nitrospirae* over MTB of the *Alphaproteobacteria* based on the racetrack method used to enrich MTB from Mickey Hot Springs.^{79,107–109} However, it should be noted that we previously used this same racetrack method to purify MTB belonging to the class *Alphaproteobacteria* from Pavilion Lake, British Columbia.⁸³ It may also be important to note that the MTB were not enriched from the water and sediment samples for a few weeks after collection from Mickey Hot Springs. This delay in enrichment could have played a role in selected for one dominant type of MTB. For example, *Nitrospirae* MTB may have been more viable in the water and sediment samples that were stored in the laboratory at room temperature prior to enrichment.^{108,109}

Discovering MTB living in a geochemically unique environment such as Mickey Hot Springs is interesting and important for several reasons. The geothermal spring has a moderately high temperature (45–50 °C) and high arsenic concentrations (\sim 1 mg/L). The MTB from Mickey Hot Springs synthesize bullet-shaped magnetite, which is notable because this is

thought to be the most ancient type of crystal biomineralized by MTB.^{39,93,94} Nitrospirae that synthesize bullet-shaped magnetite have been found globally and are the deepest branching phylum containing MTB.^{38,68,69,95,110,111} Magnetite is known to be a robust biomarker, and bullet-shaped crystals appear to be the best preserved magnetofossil type.^{55–57,62,63,68,69,93,94,112,113} It is noteworthy to point out that no MTB of the Nitrospirae have ever been isolated and grown in pure culture although several MTB from this group have been relatively well described.^{5,38,68,69}

Previous work found MTB that synthesize bullet-shaped magnetite were detected only within the oxic-anoxic transition zone (OATZ), suggesting that magnetofossil morphologies could be used to identify the OATZ in the geological record.⁵² Li et al. (2020)⁹⁴ described species-specific magnetite crystal growth in WYHR-1, a member of the phylum *Deltaproteobacteria*. WYHR-1 synthesized bullet-shaped magnetite through three crystal growth stages, which is different from how other *Deltaproteobacteria* and *Nitrospirae* MTB biomineralize magnetite crystals and suggests species-specific magnetite biomineralization.⁹⁴

Magnetite crystals preserved in the rock record as magnetofossils, and have been found in Mesozoic rocks, and may extend back as far as the pre-Cambrian.^{55–58} Differentiating between magnetite made by MTB and abiogenic magnetite in the fossil record can be challenging, but there are distinctions between the two, such as size, morphology, uniformity, chemical purity, and sometimes the magnetosome chain is preserved.^{39,53,55,57,59–69,93,94} A recent study by Liu et al. $(2021)^{42}$ found that magnetotactic cocci synthesize strainspecific magnetosome morphologies. These results suggest that magnetofossil morphology could provide valuable information regarding the paleobiology of ancient MTB for some species.⁴²

MYR-1, an uncultured MTB belonging to the phylum Nitrospirae, synthesizes bullet-shaped magnetite using a multistep process distinct to Nitrospirae.³⁸ MYR-1 have a large size (6–10 μ m in length) and synthesize up to 1000 bullet-shaped magnetosomes per cell.^{38,114} The genome of MYR-1 (tentatively named Candidatus Magnetobacterium) was sequenced and found to contain a magnetosome gene island with a novel set of genes (man genes), that may control the biomineralization of bullet-shaped magnetite.¹¹⁵ In Desulfovibrio magneticus RS-1, the biomineralization of bullet-shaped magnetite was found to grow from a solid ferrous iron precursor like green rust.⁵⁰ These findings suggest an intriguing possibility that the redox chemistry of the aquatic habitat may determine the mechanism (i.e., Fe²⁺ oxidative route versus Fe³⁺ reductive route) that MTB utilized to biomineralize bulletshaped magnetite.⁵⁰ Future studies with MTB from Mickey Hot Springs should attempt to determine the mechanism by which these bacteria synthesize bullet-shaped magnetite.

If magnetofossils can be found in sinter deposits at Mickey Hot Springs, they might facilitate the characterization of the paleoenvironment and demonstrate their utility as a biosignature for ancient magnetotactic life at the site.⁷⁷ Elongated, bullet-shaped magnetite crystals, similar to those observed in this study, would serve as an ideal biomarker at Mickey Hot Springs because such crystals are not known to form inorganically.^{38,68,69} The hydrothermal fluid at Mickey Hot Springs is supersaturated with amorphous silica¹¹⁶ making these springs ideal for mineralization processes. In fact, fossilized bacteria have been previously discovered in sinter deposits at Mickey Hot Springs.⁷⁷ In addition, the MTB that we discovered in Mickey Hot Springs produce rather unique elongated anisotropic crystals, which itself could permit the use of these biominerals as robust biomarkers for past life.^{39,59,68,69,93,94}

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ABBREVIATIONS

EPA; Environmental Protection Agency; EDX; energydispersive spectroscopy; HAADF; high angle annular dark field; MTB; magnetotactic bacteria; SEM; scanning electron microscopy; STEM; scanning transmission electron microscopy; TEM; transmission electron microscopy.

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