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Research Article

Physiology, Metabolism, and Fossilization of Hot-Spring Filamentous Microbial Mats

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Abstract

The evolutionarily ancient Aquificales bacterium *Sulfurihydrogenibium* spp. dominates filamentous microbial mat communities in shallow, fast-flowing, and dysoxic hot-spring drainage systems around the world. In the present study, field observations of these fettuccini-like microbial mats at Mammoth Hot Springs in Yellowstone National Park are integrated with geology, geochemistry, hydrology, microscopy, and multi-omic molecular biology analyses. Strategic sampling of living filamentous mats along with the hot-spring $CaCO_3$ (*travertine*) in which they are actively being entombed and fossilized has permitted the first direct linkage of *Sulfurihydrogenibium* spp. physiology and metabolism with the formation of distinct travertine streamer microbial biomarkers. Results indicate that, during chemoautotrophy and CO_2 carbon fixation, the 87–98% *Sulfurihydrogenibium*-dominated mats utilize chaperons to facilitate enzyme stability and function. High-abundance transcripts and proteins for type IV pili and extracellular polymeric substances (EPSs) are consistent with their strong mucus-rich filaments tens of centimeters long that withstand hydrodynamic shear as they become encrusted by more than 5 mm of travertine per day. Their primary energy source is the oxidation of reduced sulfur (*e.g.,* sulfide, sulfur, or thiosulfate) and the simultaneous uptake of extremely low concentrations of dissolved O_2 facilitated by bd-type cytochromes. The formation of elevated travertine ridges permits the *Sulfurihydrogenibium*-dominated mats to create a shallow platform from which to access low levels of dissolved oxygen at the virtual exclusion of other microorganisms. These ridged travertine streamer microbial biomarkers are well preserved and create a robust fossil record of microbial physiological and metabolic activities in modern and ancient hot-spring ecosystems. Key Words: Filamentous microbial mats—Hot-spring—Travertine—Biomarkers—*Sulfurihydrogenibium.* Astrobiology 19, xxx–xxx.

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1. Introduction

NASA's highly interdisciplinary *Astrobiology Roadmap* and its recent update called the *Astrobiology Strategy* present the scientific foundations required for effective ongoing and future studies of the origin, evolution, composition, and distribution of life throughout the Universe (Des Marais *et al.,* 2008; Hays, 2015). A fundamental component of these efforts is to understand the coevolution and fossilization of microbial life within the context of changing habitable environmental conditions on early Earth and potentially other planets. Microbes initially survive by utilizing and responding to the physical, chemical, and biological parameters of the environments they originated in and colonized. Successful adaptation and evolution can eventually lead these microorganisms to alter and ultimately control these same environmental conditions. Deciphering and predicting these complex feedback processes requires in-depth geology and biology (*geobiology*) knowledge of the interwoven abiotic and biotic factors that control mineral precipitation, sedimentary rock deposition, and the resulting formation of microbial fossils (*biomarkers*). To do this, studies of microbial biomarker formation in modern-day terrestrial hot springs on Earth are used to more accurately identify ancient microbial biomarkers and reconstruct the paleoenvironmental conditions they once inhabited (Roling *et al.,* 2015).

Mammoth Hot Springs (MHS) in Yellowstone National Park (YNP; Fig. 1) has long been a strategically important natural laboratory in which to determine how microbial biomarkers form (Fouke *et al.,* 2000, 2003; Fouke 2001, 2011; Kandianis *et al.,* 2008; Veysey *et al.,* 2008). Hot-spring calcium carbonate ($CaCO_3$) limestone (*travertine*) is rapidly deposited at MHS to entomb heat-loving (*thermophilic*) filamentous microorganisms of the order Aquificales. *Sulfurihydrogenibium* spp., the most abundant of the Aquificales at MHS, exhibits physiological plasticity and catalyzes the growth of $CaCO_3$ (*aragonite*) crystals to rapidly precipitate thick deposits of sinuous (*streamer*) travertine. Aquificales is a deeply-rooted evolutionary lineage of the bacteria that thrives in high-temperature, fast-flowing, and dysoxic turbulent water in thermal systems throughout YNP and around the world (Blank *et al.,* 2002; Nakagawa and Fukui, 2003; Aguiar *et al.,* 2004; Ferrera *et al.,* 2007; Purcell *et al.,* 2007; Flores *et al.,* 2008; O'Neill *et al.,* 2008; Inskeep *et al.,* 2013).

Previous studies at MHS have established an extensive quantitative baseline of the physical, chemical, and biological conditions in which *Sulfurihydrogenibium*-dominated filamentous microbial mats rapidly grow and simultaneously become encrusted to form travertine streamers (Reysenbach *et al.,* 2009; Inskeep *et al.,* 2010, 2013; Fouke, 2011; Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). These travertine streamers are composed of (1) fine-scale crystalline preservation of the *Sulfurihydrogenibium*-dominated filamentous mats that accurately record spring water temperature, pH, and flux; (2) well-preserved lipid biomarker signatures reflecting their sulfur cycling metabolism; and (3) direct catalytic influence on the rate of travertine precipitation (Fouke, 2011). However, several fundamental questions have remained unanswered regarding the specific metabolic pathways utilized by *Sulfurihydrogenibium* for energy and nutrient assimilation, and how the crystalline record of these processes are preserved and reflected during travertine streamer biomarker formation. The present study documents a direct linkage between *Sulfurihydrogenibium* physiology and metabolism with resulting travertine microbial biomarker formation. This is accomplished by integrating field observations and analyses of hydrology, aqueous chemistry, and sedimentary petrography (travertine crystalline structure, geochemistry, and geomorphology) with next-generation metagenomic, metatranscriptomic, and metaproteomic approaches. Results establish that fossilized ridged travertine streamer microbial biomarkers sensitively and accurately preserve the physiological and metabolic activities, as well as the environmental conditions, of hot-spring filamentous microbial mats.

2. Geobiological Setting

Previous long-term studies at MHS have quantitatively characterized the CO_2-rich Ca-Na-HCO_3-SO_4 spring water geochemistry, travertine deposition, and microbial community diversity along the hot-spring outflow drainage systems (Fouke, 2011). Temperature and pH measurements in the context of a travertine depositional facies model (described below) at Narrow Gauge Spring within the MHS thermal complex (Fig. 1) were used here to correlate with these previously published analyses. This permitted a comprehensive environmental contextualization of the *Sulfurihydrogenibium*-dominated filamentous microbial mats analyzed in the present study (Fouke *et al.,* 2003; Inskeep *et al.,* 2010, 2013; Fouke, 2011; Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013) (Supplementary Table S1; Supplementary Information is available online at www.liebertonline.com/ast).

The MHS drainage systems are composed of a sequence of five distinct types of upstream-to-downstream travertine rock deposits (*facies*) composed of unique crystal sizes and shapes, mineralogies, chemistries, and geomorphologies (Fouke *et al.,* 2000; Fouke, 2011). Each of these facies rock deposits reflects a specific combination of physical, chemical, and biological processes that were active in the hot-spring environment that formed each unique facies. The composition and succession of each travertine facies, including the Apron and Channel Facies (ACF) that is the focus of the present study, is consistently reestablished after the hot-spring vents shift their position, close, or reemerge elsewhere in the MHS system (Fouke *et al.,* 2000; Fouke, 2011). As a result, the crystalline fabric and geomorphology of modern and ancient ACF travertine deposits can be used to accurately and reproducibly reconstruct temperature, pH, hydrology and other environmental factors (Veysey *et al.,* 2008; Fouke, 2011). The MHS travertine facies model has also been proven to be directly applicable to other modern and ancient travertine-depositing hot-spring ecosystems around the world (Fouke 2001; Veysey *et al.,* 2008).

The present study focuses on the high-temperature (65–72°C) and low-pH (6.2–6.8) environment where sinuous ACF travertine deposits are formed when *Sulfurihydrogenibium*-dominated microbial mat streamers are encrusted by densely packed radial bundles of needle-like (*acicular*) $CaCO_3$ aragonite crystals (Fig. 1) (Fouke *et al.,* 2000; Fouke, 2011). Here, travertine mimics the morphology of the ACF filamentous microbial mats, which forms a well-preserved travertine

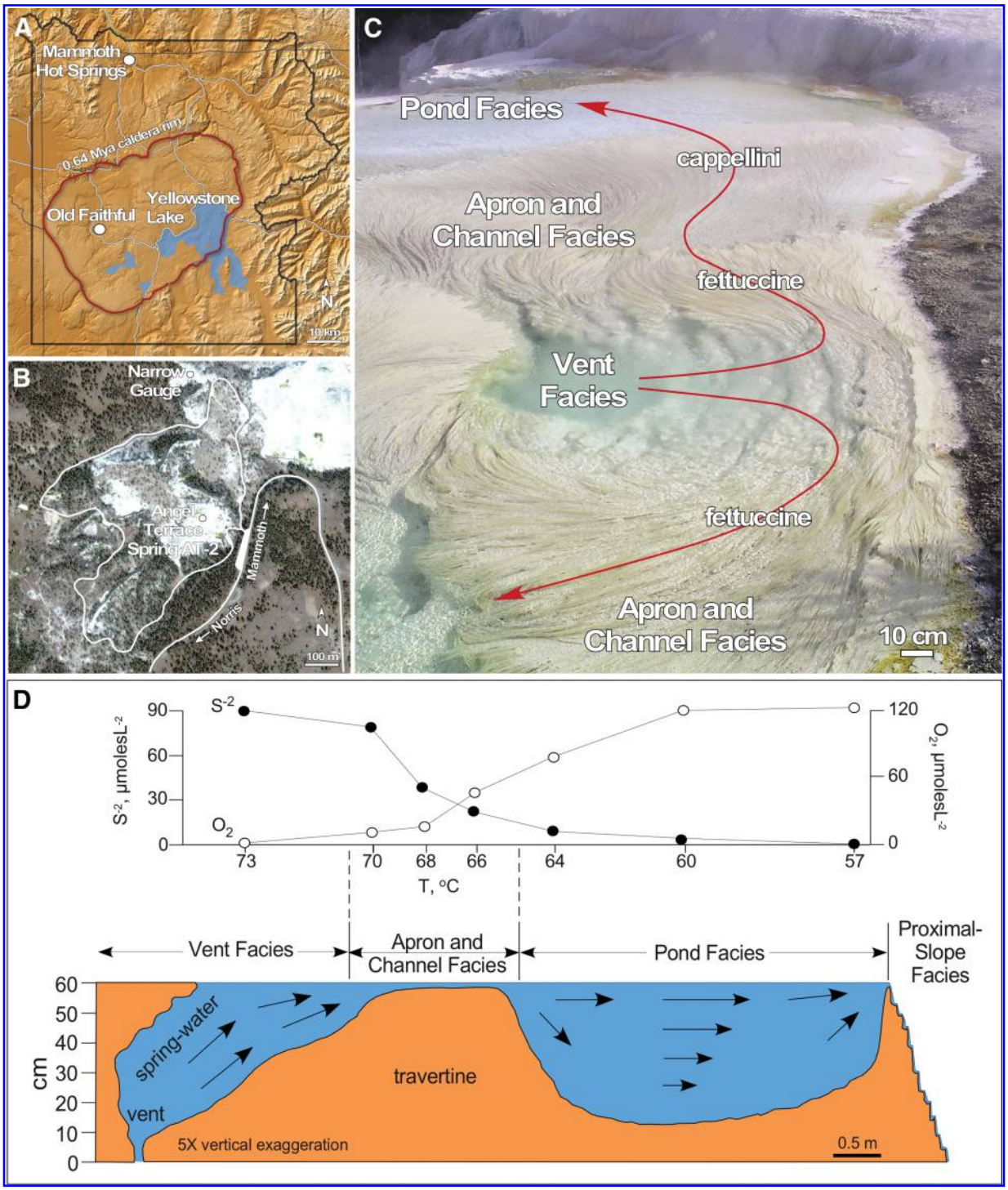

FIG. 1. (**A**) Map of Yellowstone National Park. (**B**) Satellite image of MHS. (**C**) Field photograph overview of a spring outflow at MHS. (**D**) Cross section of the Vent, Apron and Channel, and Pond Facies with quantified physical and chemical conditions. B, C, and D are modified from Fouke (2011) and Fouke and Murphy (2016).

microbial biomarker (Veysey *et al.,* 2008). The upstream 69–72°C portion of the ACF depositional environment forms pasta-like travertine deposits called the ''fettuccini'' streamer fabric (Fig. 1). In these locations, bundles of intertwined microbial filaments can reach 2 mm in diameter and 10 cm in length (Fouke, 2011). Aragonite travertine entombment is minimal at the downstream growing tips of each horizontal filament and rapidly increases upstream along each filament (Figs. 2 and 3). A suite of reduced sulfur compounds including sulfide (HS^- aqueous), thiosulfate ($S_2O_3^{2-}$ aqueous), sulfur (S^0 solid), reduced arsenic species and dissolved inorganic carbon (DIC) are important geochemical components in these upstream portions of the MHS drainage systems (Fouke, 2011).

Molecular microbial ecology studies of the ACF fettuccini streamer travertine depositional environments consistently indicate they are composed of stable and relatively low-diversity

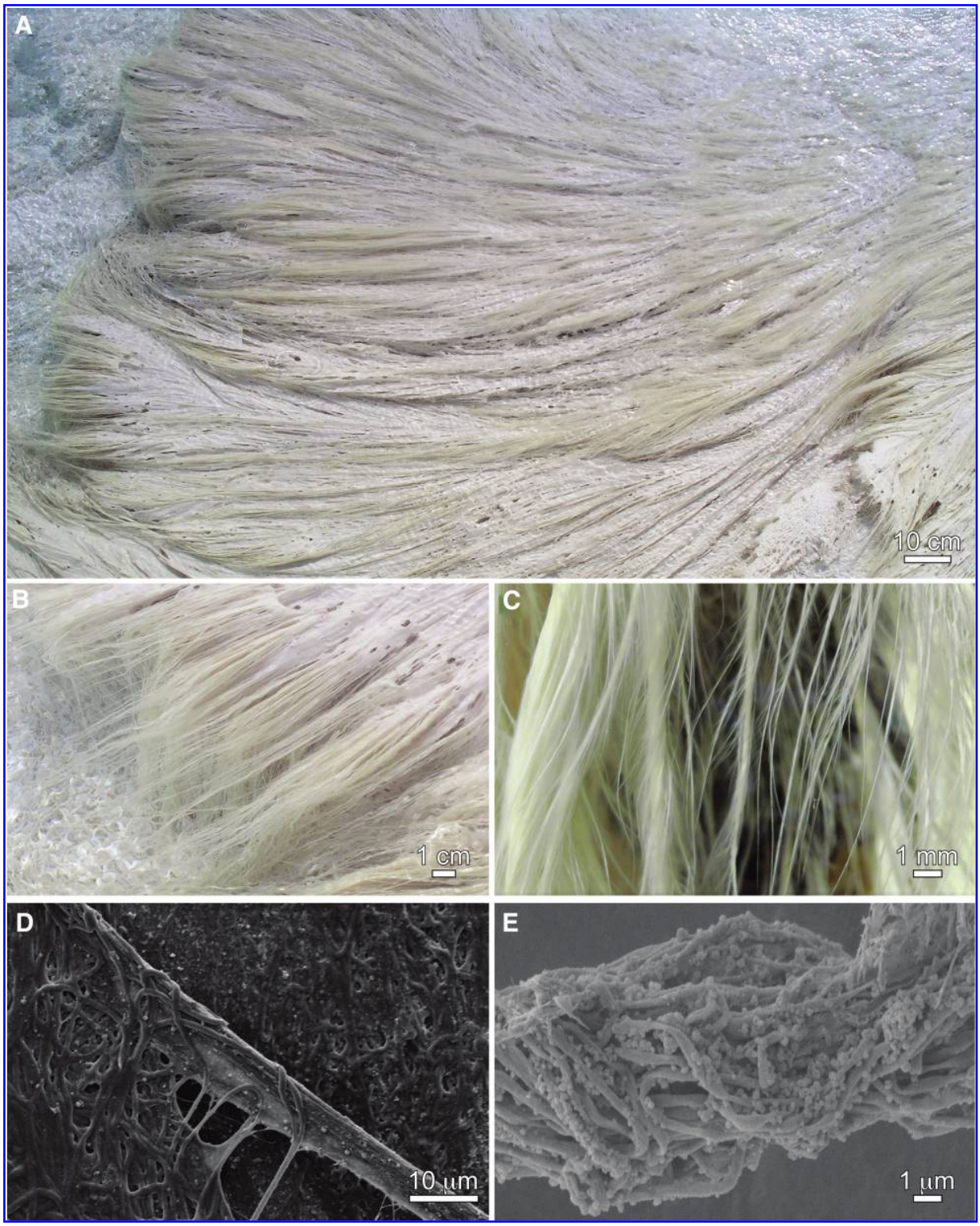

FIG. 2. *Sulfurihydrogenibium*-dominated filamentous microbial mats inhabiting the ACF. (**A**) Field photograph of the fettuccini streamer travertine. (**B** and **C**) Naked microbial filaments not yet encrusted by travertine. (**D** and **E**) Scanning electron photomicrographs of the naked microbial filaments. A, B, and E are modified from Fouke (2011) and Fouke and Murphy (2016).

microbial communities dominated by *Sulfurihydrogenibium* spp. (Fouke *et al.,* 2003). Recent metagenomic and metatranscriptomic analyses of *Sulfurihydrogenibium* isolates from YNP have substantiated this community structure and permitted reconstruction of their basic metabolic strategies (Reysenbach *et al.,* 2009; Inskeep *et al.,* 2010, 2013; Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). This work has shown that *Sulfurihydrogenibium* spp. are chemolithoautotrophic sulfur oxidizers that fix CO_2 using the reductive reversed tricarboxylic acid (rTCA) cycle. *Sulfurihydrogenibium azorense* and

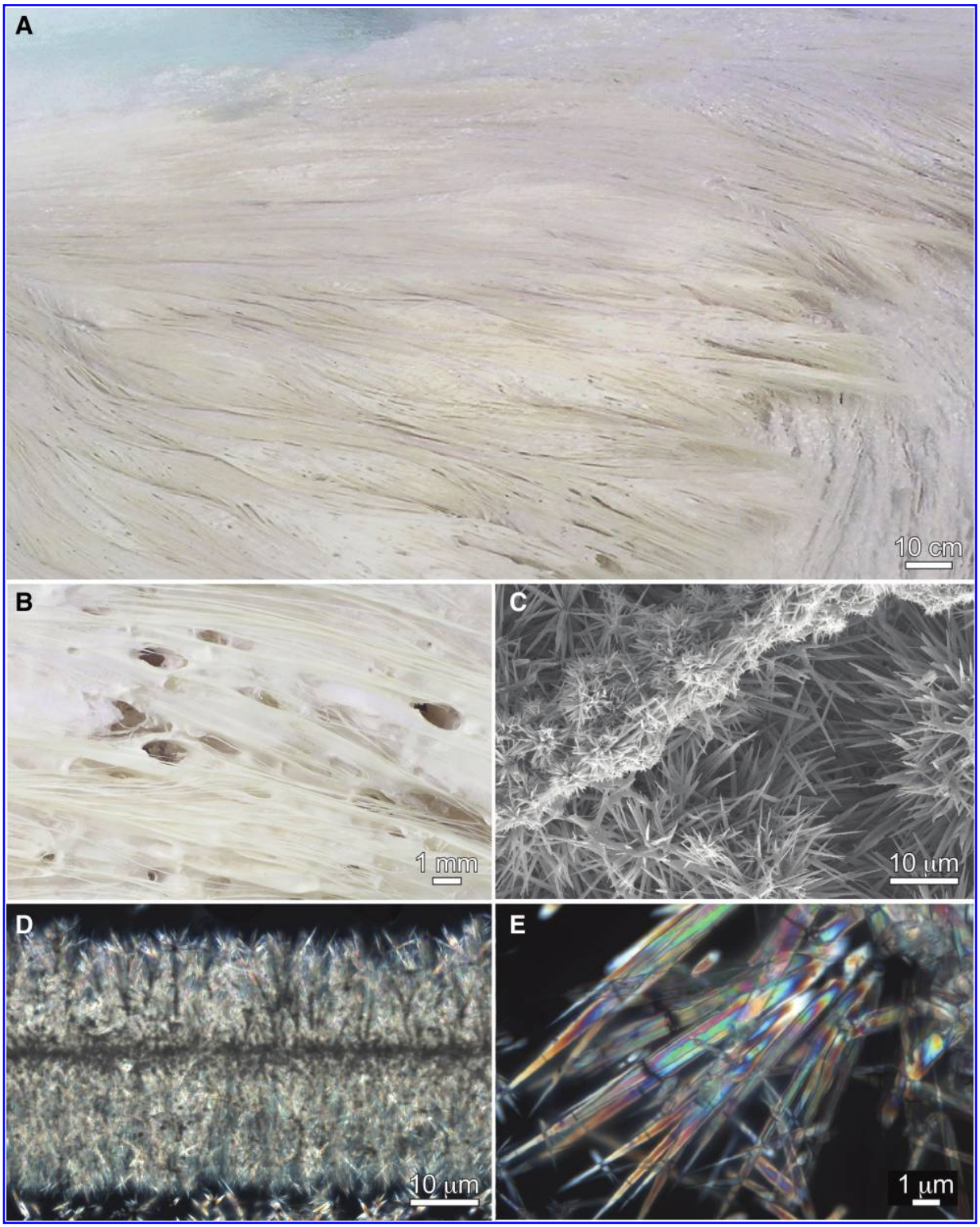

FIG. 3. *Sulfurihydrogenibium*-dominated filamentous microbial mats inhabiting the ACF. (**A**) Field photograph of the fettuccini streamer travertine. (**B**) Elliptical holes torn in the sheets of EPSs draped between filaments. (**C**) Scanning electron photomicrographs of small aragonite needles encrusting microbial filaments and large aragonite needles encrusting interfilament EPSs. (**D** and **E**) Polarized bright-field photomicrograph of aragonite needles encrusting a microbial filament. C, D, and E are modified from Fouke (2011) and Fouke and Murphy (2016).

S. kristjanssonii (Aguiar *et al.,* 2004; Flores *et al.,* 2008) contain Group 1 Ni-Fe hydrogenases and can oxidize hydrogen. However, these genes were absent in the metagenomes of ACF streamer communities at MHS (Inskeep *et al.,* 2010; Takacs-Vesbach *et al.,* 2013). This observation, coupled with the absence of Group I Ni-Fe hydrogenases in the *Sulfurihydrogenibium yellowstonense* genome (Reysenbach *et al.,* 2009), and the inability of this organism to grow on hydrogen, suggests that these microbial communities utilize other electron donors to drive the fixation of inorganic carbon.

3. Materials and Methods

Detailed description of all protocols and techniques used for field collection, biomolecule extractions, and meta-omic analyses are presented in the Supplementary Information and briefly summarized here. Detailed descriptions of the experimental design and metadata curation strategies adopted for all aspects of the field and laboratory analyses in the present study are presented in the works of Palmer *et al.* (2017) and Thomer *et al.* (2018).

3.1. Yellowstone field sample collection

Filamentous microbial mat samples were collected from ACF fettuccini streamer travertine depositional environments (Fig. 2) at Narrow Gauge Spring (44°54′17.46″N, 110°24′14.5″W) in the MHS thermal complex. Quantitative physical, chemical, and biological measurements of the environmental conditions in which the ACF travertine within the MHS facies model is deposited have previously been published (Fouke *et al.,* 2003; Inskeep *et al.,* 2010, 2013; Fouke, 2011; Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). In addition to the streamer travertine morphology and crystalline structure, the precise correlation with previously published ACF environmental parameters was confirmed with measurements of temperature and pH using an Orion Model 290A probe (Veysey *et al.,* 2008). Filamentous microbial mat samples were collected from the most distal portions of the ACF fettuccine streamer travertine depositional environment (Fig. 2A, 2B) and shipped overnight in a –80°C Taylor-Wharton CX100 dry shipper liquid nitrogen dewar to the Carl R. Woese Institute for Genomic Biology at the University of Illinois at Urbana-Champaign.

3.2. Nucleic acid extraction and analyses

Genomic DNA and RNA were extracted in the Carl R. Woese Institute for Genomic Biology from the centrifuged pellets by using a procedure modified from Dong *et al.* (2014) and Inskeep *et al.* (2010). One gram of microbial mat pellet was derived from each of two separate replicate samples collected from the spring, pooled, and mixed for extraction. Frozen samples were thawed on ice and centrifuged at 9000 rpm on a SORVALL Legend RT centrifuge (Fisher Thermo Scientific Inc., MA) at 4°C for 20 min, after which the supernatant was carefully decanted. The remaining pellets were incubated for 0.5 h with 2 mL of lysis buffer (0.15 M NaCl, 0.1 M Na_2EDTA, pH = 8.0) containing 15 mg/mL lysozyme (Sigma-Aldrich, MO) at 37°C. This step was repeated once, and the supernatant for the two rounds of lysis was decanted and pooled (Inskeep *et al.,* 2010). The supernatant was then incubated with equal amount of SDS-Tris solution (0.1 M NaCl, 0.5 M Tris-Cl, pH = 8.0, 10% sodium dodecyl sulfate). Freeze-thaw cycling between a 55°C water bath and liquid nitrogen was performed three more times to ensure further cell wall lysis, followed with incubation for 0.5 h with proteinase K (100 μg/mL). Phenol:chloroform extractions and precipitation were performed following the methods presented in the work of Dong *et al.* (2014). Samples were placed in 100 μL TE buffer (pH 7.4), with the quality and quantity of recovered DNA and RNA confirmed by gel electrophoresis. The extracted DNA and RNA were then loaded onto a 1.0% agarose gel and stained with ethidium bromide. The size and intensity of the genomic DNA and RNA bands were compared visually with a quantitative DNA ladder (Hyperladder I, Bioline USA, Boston, MA).

3.3. Protein and peptide extraction and analyses

Metaproteomic analyses of the ACF fettuccini streamer microbial community were conducted by using multiple extraction and analysis methods at the Division of Glycoscience, Royal Institute of Technology (KTH), Sweden, and the Roy J. Carver Biotechnology Center, University of Illinois Urbana-Champaign. The protein extractions were performed on the streamer microbial mat samples that had been thawed at 4°C. Four different extraction protocols and two protein analysis methods were used in the independent Illinois and Stockholm laboratories to obtain and compare protein and peptide recovery, thus permitting the potential bias of any individual methodology (described in the Supplementary Information).

The peptides simultaneously matching two or more proteins (non-unique) were excluded from the following analyses. A protein was considered as ''identified'' when it had at least one unique peptide hit, and those with no unique peptides (*e.g.,* only identified by shared peptides) were excluded. In addition, only the proteins, which contained at least two unique peptides from one method or were identified in no less than two different extraction/analysis methods, were used for the following analyses. The protein identifications were exported with a protein probability p value (Mascot) of greater than 0.05 (peptide 0.95). The false discovery rate was 9–21%, which is high due to the limited comparative data base for natural hot-springs and the short length of the transcriptomic reads. However, all protein identification inferences were consistently corroborated by both the protein probability p value and metagenomic analyses. The relative abundance of each identified protein was calculated by using normalized spectral abundance factor, which considered both abundance and size of the protein (Zybailov *et al.,* 2006). The taxonomy and annotation of the identified proteins were retrieved from the open reading frames (ORFs) predicted from the metagenome of the same ACF fettuccini microbial community (as described above). The proteins detected from the libraries by using four different extraction methods were pooled for identification of unique proteins. This was then integrated with the results from metagenomic and metatranscriptomic analyses for metabolic reconstruction.

3.4. Bioinformatic and statistical analyses

Bioinformatic analyses were completed at the Roy J. Carver Biotechnology Center at the University of Illinois Urbana-Champaign. Metagenome assembly and phylogenetic

binning were performed as described in the Supplementary Methods. Comparative genomic analyses were performed with OrthoVenn (Wang *et al.,* 2015). ORFs assigned to the *Sulfurihydrogenibium* were extracted for metabolic reconstruction and predicted by using IMG/MER's native collection of pathways (Markowitz *et al.,* 2012). These were manually curated based on available literature about Aquificales and their associated microbial communities in thermal and hyperthermal environments. Clusters of orthologous groups of proteins (COGs) that were significantly enriched or over-presented in cDNA dataset relative to DNA dataset at 0.95 confidence level were identified with XIPE, a nonparametric, difference-of-medians analysis method (Rodriguez-Brito *et al.,* 2006). Only the metabolic pathways containing ORFs with enriched transcription as well as expressed proteins were considered.

3.5. Data accession

The reads of the V3–V5 hypervariable region of the 16S rRNA genes are available in the Short Reads Archive (SRA; SRR4444881). The raw sequencing of reads and assembled contigs for the metagenomic and metatranscriptomic libraries of the ACF fettuccini streamer microbial community are available in the SRA (SRR4436414–SRR4436415) and Whole Genome Shotgun (WGS) under accession number (SRX2255526, SRX2255527). The genomes of the dominant *Sulfurihydrogenibium* spp. are available in the IMG/ER under the IMG Submission ID 79768. Mass spectrometry proteomics data has been deposited in the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD004323 and PXD006504–PXD006506.

4. Results

Field observations, optical microscopy, and scanning electron microscopy for the MHS ACF depositional environment have previously been published (Fouke *et al.,* 2000; Kandianis *et al.,* 2008; Veysey *et al.,* 2008; Fouke, 2011). Our newly collected metagenomic, metatranscriptomic, and metaproteomic analyses suggest that filamentous mats growing within the ACF depositional environment at Narrow Gauge Spring are dominated by *Sulfurihydrogenibium* spp. (Supplementary Information). Fully 87% of the metagenomic reads and 88% of the metatranscriptomic libraries align with the *Sulfurihydrogenibium* pangenome B001 (Figs. 4–6). This pangenome is effectively a metagenome assembled genome. Metaproteomic results indicate that 533 out of 665 unique proteins were associated with the pangenome (Fig. 6). The metaproteomic library, constructed by using combined sodium dodecyl sulfate

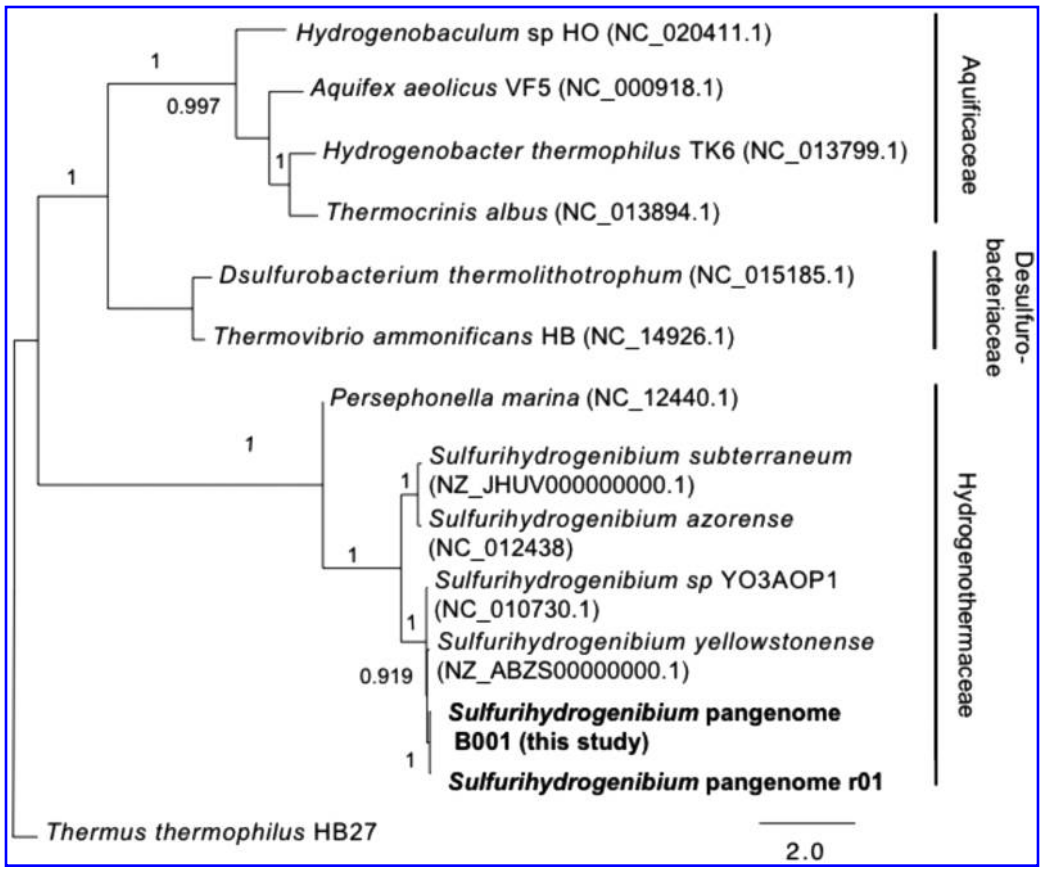

FIG. 4. A phylogenomic tree reconstruction based on available pangenomes and genomes (phylogenomic trees) related to the *Sulfurihydrogenibium* spp.–dominated filamentous microbial mats analyzed in the present study. *Sulfurihydrogenibium* pangenome B001 and the closely related Aquificales. *Sulfurihydrogenibium* pangenome r01 are the terms used for the curated *Sulfurihydrogenibium* pangenome reconstructed from the metagenomes of previously reported microbial communities associated with the ACF depositional environment at MHS (Inskeep *et al.,* 2010, 2013; Takacs-Vesbach *et al.,* 2013). The scale bar indicates genetic substitutions of 2% per unit of the sequence length. Values next to the nodes are pping values. Identification numbers in parentheses are the genome accession numbers shown in the NCBI bacterial genome database.

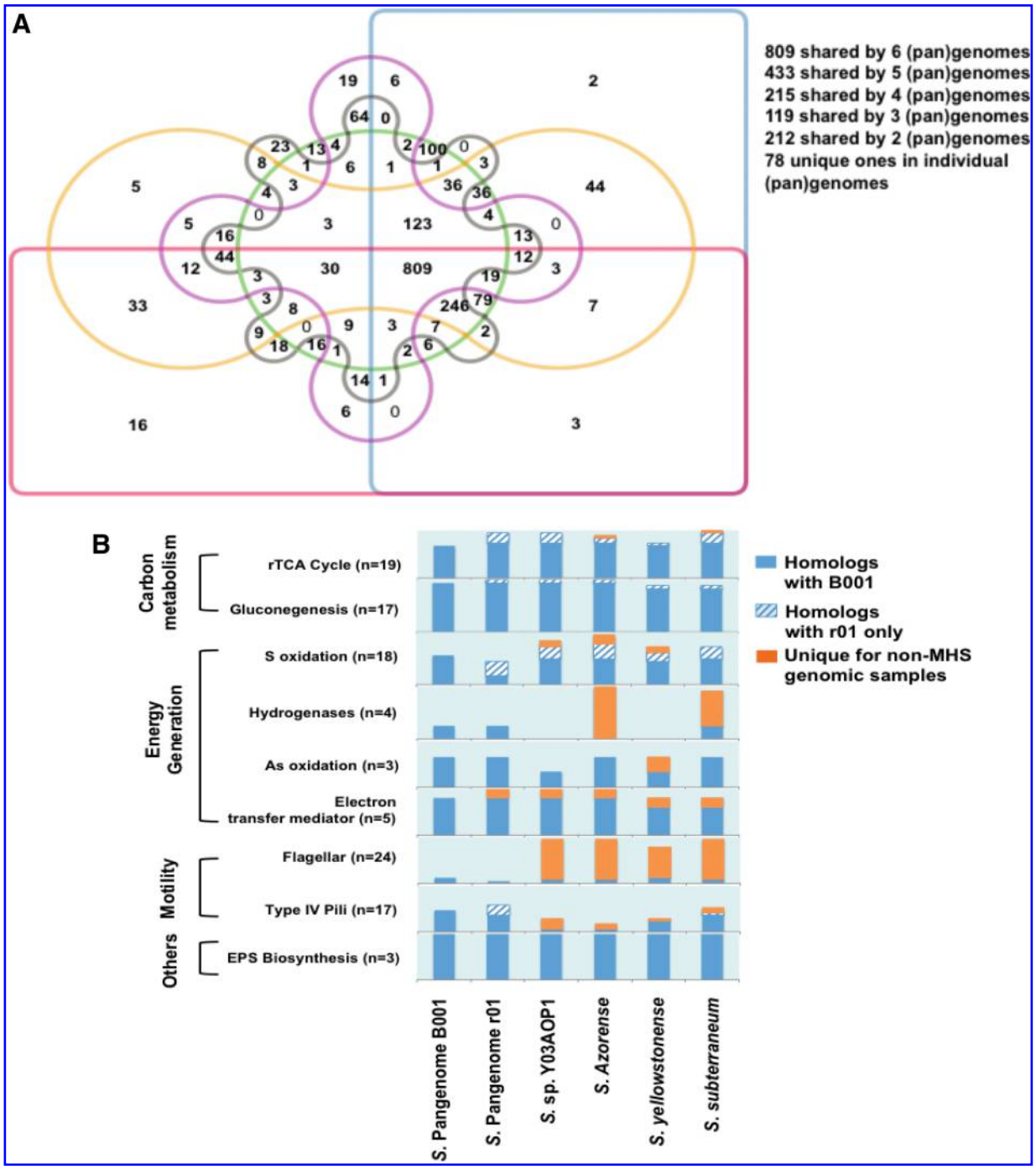

FIG. 5. Comparative genomic analyses (**A**) and gene distribution for individual metabolic function (**B**) for the *Sulfurihydrogenibium* pangenomes and the known *Sulfurihydrogenibium* genomes. In A, the annotation was conducted with IMG, and the metabolic pathways were determined with KEGG (Markowitz *et al.,* 2012). These comparative genomic analyses were conducted using OrthoVenn (Wang *et al.,* 2015) with an e-value of $1e^{-10}$ and inflation value 1.5. The values indicated the number of protein clusters shared between the *Sulfurihydrogenibium* pangenomes and genomes; those highlighted in red meant the number of protein clusters unique for the MHS-YNP ACF *Sulfurihydrogenibium* pangenomes but absent in the known *Sulfurihydrogenibium* genomes. In B, the fractions in percentile were calculated by dividing the number of protein-coding genes affiliated to a specific metabolic function for individual genome/pangenome by the nonredundant value for those of all the *Sulfurihydrogenibium* genome and pangenomes under investigation. The values in parentheses indicated the total number of genes counted for each metabolic function with the detailed information listed in Table S4.

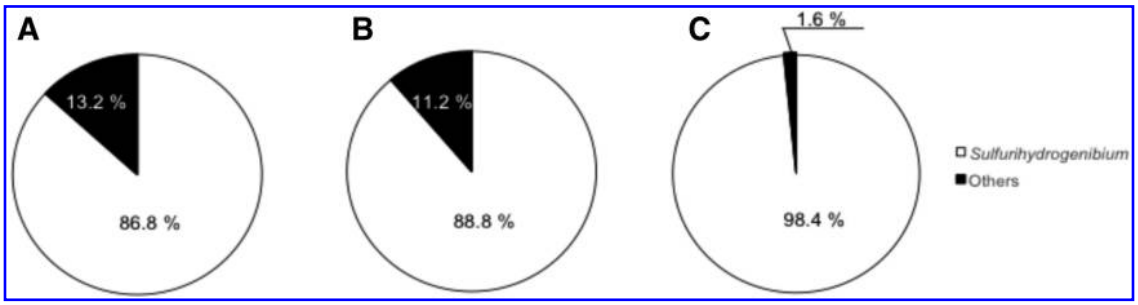

FIG. 6. Dominance of *Sulfurihydrogenibium* spp. in the filamentous microbial communities based on taxonomic assignments of the non-rRNA genes, mRNA transcripts, and proteins in the metagenomic (**A**), metatranscriptomic (**B**), and metaproteomic (**C**) libraries showed. The values listed indicate relative abundance in percent. Proteomic analyses indicate proteins recovered using integrated SDS lysis and in-gel digestion as the reference library for three pooled protein extraction methods.

(SDS) lysis and in-gel digestion extraction methods (Supplementary Information), has 98.4% of its peptides assigned to the *Sulfurihydrogenibium* pangenome B001 (Figs. 6 and S1, Table S4). The *Sulfurihydrogenibium* pangenome B001 functional profile is built upon COGs (Tatusov *et al.,* 2000) of functional protein categories determined for genes, transcripts, and proteins from the metagenomic, metatranscriptomic, and metaproteomic libraries (Table 1; Fig. 5). In comparison, the relative abundance of the sequences in these libraries across 19 COG categories was more evenly distributed in the metagenome (0.53–10.46%) than in the metatranscriptomic library (0.35–27.81%) and proteomic library (0.29–29.66%) as expected (Figs. 6 and S4). The COG categories ''C-energy production and conversion,'' ''O-post-translational modification,'' and ''N-Cell motility'' were significantly over-represented in the metatranscriptomic and metaproteomic libraries (Fig. 7).

Further evaluation of the protein classes affiliated with all COG categories with bootstrapping analyses (Rodriguez-Brito *et al.,* 2006) indicated that a total of 17 protein classes have elevated expression levels for the dominant *Sulfurihydrogenibium* pangenome (Fig. 7). This is shown by their significant enrichment in the metatranscriptomic library relative to the metagenomic library (95% confidence level; Table 1). Analyses of these over-represented protein classes showed that the majority of them corresponded to the most abundant ($n=40$) transcripts in terms of fragments per kilobase of exon per million fragments mapped (FPKM). The cognate proteins were also identified for most of these over-represented protein-coding genes (Fig. 7). Evaluation of the over-represented genes and cognate proteins showed that they were closely associated with nutrient metabolism (*e.g.,* rTCA cycle), energy generation (*e.g.,* sulfur oxidation) and tolerance of environmental stresses (*e.g.,* chaperonins, putative cold shock proteins, type IV pili, and EPS biosynthesis) by the *Sulfurihydrogenibium* spp. (Fig. 8; Tables S6–S10). Importantly, comparative genomic analyses reveal that the MHS *Sulfurihydrogenibium* has several genetic traits that are distinct from those communities inhabiting other geographically isolated geothermal environments (Reysenbach *et al.,* 2009). In addition, differences are observed in the functional genes comprising the two MHS pangenomes (Fig. 4, Table S6).

Table 1. COGs That Are Significantly Enriched in the cDNA Dataset Relative to the DNA Dataset at 0.95 Confidence Level

COG Cluster	*Representation in cDNA (%)*[a]	*Representation in DNA (%)*
Succinyl-CoA synthetase, beta subunit (COG0045)	9.58e-1	1.90e-3
GTPases—translation elongation factors (COG0050)	3.29	1.67e-3
Succinyl-CoA synthetase, alpha subunit (COG0074)	3.50e-1	6.34e-4
Co-chaperonin GroES (HSP10) (COG0234)	1.01	2.24e-4
Glycosyltransferase (COG0438)	9.38e-2	5.42e-3
Chaperonin GroEL (HSP60 family) (COG0495)	1.03	9.20e-4
Succinate dehydrogenase/fumarate reductase, Fe-S protein subunit (COG0479)	5.41e-1	7.16e-4
Translation elongation factors (COG0480)	9.83e-1	1.64e-3
Pyruvate:ferredoxin oxidoreductase or related 2-oxoacid:ferredoxin oxidoreductase, alpha subunit (COG0674)	5.79e-1	7.49e-4
NADH dehydrogenase, FAD-containing subunit (COG1252)	1.43	1.24e-3
Cytochrome bd-type quinol oxidase, subunit 1 (COG1271)	2.83	2.54e-3
Cytochrome c, mono- and diheme variants (COG2010)	6.09e-1	1.30e-3
Cation transport ATPase (COG2217)	8.98e-2	5.78e-3
Monomeric isocitrate dehydrogenase (COG2838)	1.64	2.27e-3
Cytochrome c551/c552 (COG4654)	8.93e-1	3.74e-4
Tfp assembly protein FimT (COG4970)	1.12	9.69e-4

[a]Significantly enriched in cDNA sample at the 0.95 confidence level based on the bootstrapping statistic method (Rodriguez-Brito *et al.,* 2006).

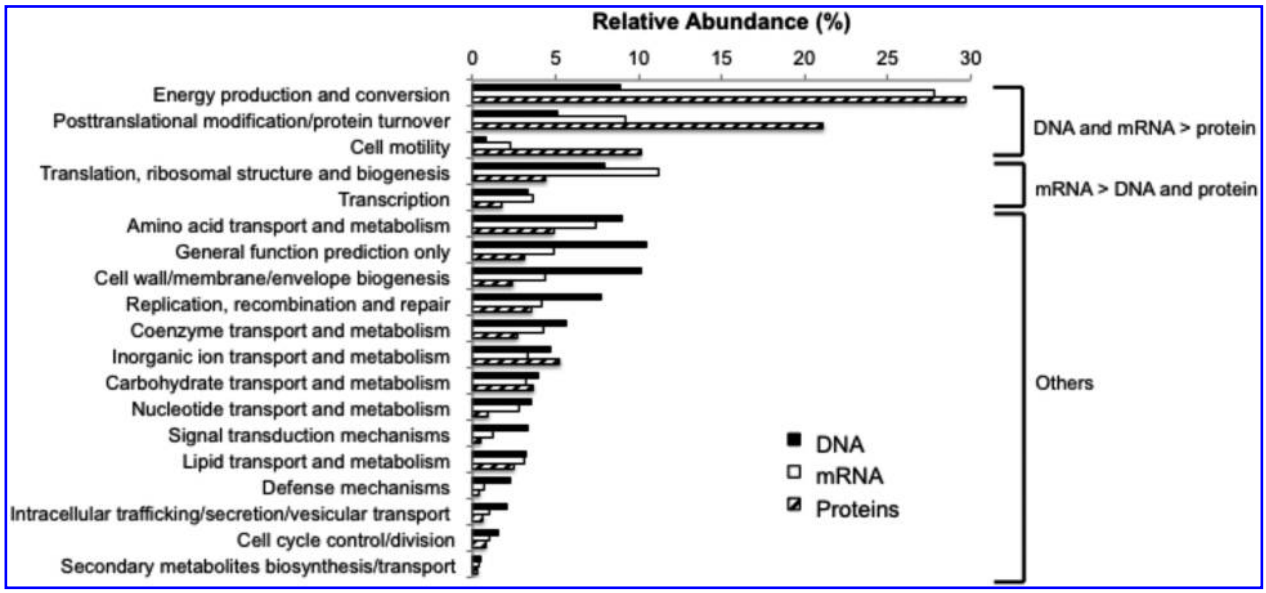

FIG. 7. Gene expression profiles at DNA, mRNA transcript, and protein levels based on the COG protein family database for the dominant *Sulfurihydrogenibium* spp. The cutoff value for the COG categories under consideration was 0.1%. The dataset from protein analyses was from the proteomic library developed on the combined SDS lysis and in-gel digestion method.

5. Discussion

5.1. Sulfurihydrogenibium *pangenome structure and diversity*

The *Sulfurihydrogenibium* pangenome B001 detected in the present study represents multiple closely related microorganisms based on repeated single-copy genes. Only one 16S rRNA gene sequence at nearly full length is observed in the present study, compared with the detection of four 16S rRNA gene sequences previously observed in *Sulfurihydrogenibium* pangenome r01 (Inskeep *et al.,* 2010, 2013; Takacs-Vesbach *et al.,* 2013). While this may in part be due to the clustering of sequences at the 97% sequence identity level (Miller *et al.,* 2011), it may also imply that the low-diversity ACF filamentous mats have an unexpectedly diverse genome structure. This pangenome comparison further suggests that protein-coding genes for carbon metabolism and extracellular polymeric substance (EPS) biosynthesis are relatively conserved. In contrast, while all *Sulfurihydrogenibium* pangenomes contain functional genes for sulfur oxidation, differences have been reported in their capacity to metabolize different sulfur-containing substrates (Takai *et al.,* 2003; Aguiar *et al.,* 2004; Nakagawa *et al.,* 2005; Flores *et al.,* 2008; O'Neill *et al.,* 2008). In addition, the two ACF *Sulfurihydrogenibium* pangenomes lack nearly all the functional genes associated with biosynthesis and assembly of flagella (Fig. 5 and Table 1), which are expressed in all the other known *Sulfurihydrogenibium* isolates (Takai *et al.,* 2003; Aguiar *et al.,* 2004; Nakagawa *et al.,* 2005; Flores *et al.,* 2008; O'Neill *et al.,* 2008; Reysenbach *et al.,* 2009). Another distinct difference is that genes associated with type IV pili are present in the MHS ACF pangenome yet absent in *Sulfurihydrogenibium* genomes from other geochemically distinct hot-springs in other geographic locations (Fig. 4 and Table S6).

While the ACF filamentous microbial mat communities are 90% distinct from those detected in the other four upstream-to-downstream travertine facies depositional environments at MHS (Fouke *et al.,* 2003), pangenome comparisons imply that there is also strain-level genetic diversity between similar *Sulfurihydrogenibium* spp. The suite of genes encoding sulfur-oxidizing proteins (*i.e., soxABXYZ*) that catalyze the oxidation of thiosulfate to sulfate is only expressed in analyses from the present study. These genes were not previously detected in *Sulfurihydrogenibium* pangenome r01 (Table S2; Inskeep *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). Furthermore, the sulfide:quinone oxidoreductase and sulfide dehydrogenase predicted in *Sulfurihydrogenibium* pangenome r01 appear to be substituted with a putative sulfide-quinone reductase with relatively low homology in the newest pangenome (Tables S2 and S6). These *Sulfurihydrogenibium* pangenomes are not complete, and gaps between scaffolds exist. However, their dominance in the microbial communities and the more than 98% completeness of the reconstructed pangenomes imply that the absence of a whole suite of functional genes (*e.g., soxABXYZ*) in the *Sulfurihydrogenibium* pangenome r01 is likely not from the limited coverage in sequencing or assembly. Alternatively, this may be due to the physical and geochemical gradient within the ACF itself and thus reflect spatial population diversity on a relatively small scale.

With respect to sulfur compounds, the concentration of sulfide decreases from about 90 to 20 μM from upstream to downstream in the ACF, while the thiosulfate ranges from 45 to 90 μM with the peak concentration in the intermediate zone (Fig. 1; Fouke *et al.,* 2003; Fouke, 2011). Sampling for the present study was completed from flowing springwater with lower pH but higher sulfide concentrations compared to sampling conducted in previous work (Inskeep *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). Previous analyses of four upstream-to-downstream sites within the ACF detected several different *Sulfurihydrogenibium* 16S rRNA phylotypes (Table S5). The dominant *Sulfurihydrogenibium* phylotype identified in the current study is most similar to those detected in the upper reaches of the ACF (fettuccini microbial community; Table S5), which is consistent with the location in which samples

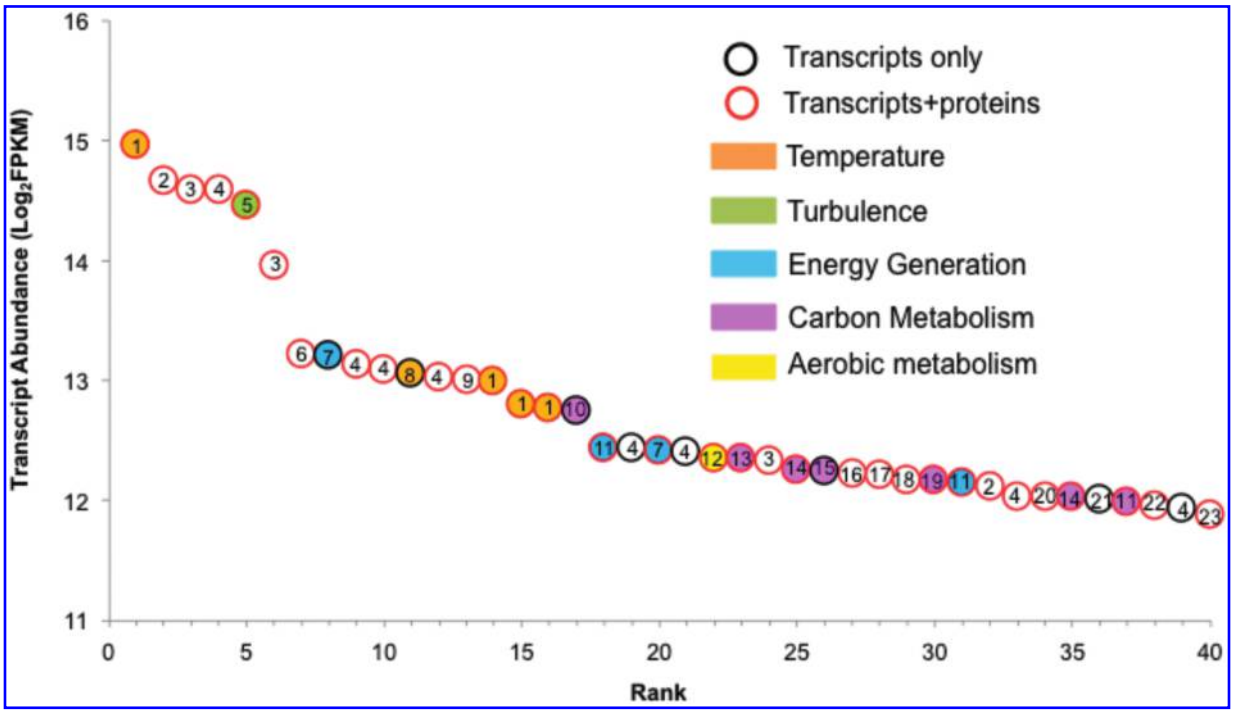

FIG. 8. The abundance of the top 40 expressed genes for the dominant *Sulfurihydrogenibium* spp. The transcript abundance was illustrated as the fragments per kilobase of transcript per million (FPKM) at logarithmic scale at the order of 2. The genes with both identified transcripts and cognate proteins were highlighted in red circles; the ones with only identified transcripts were shown in black circles. The circles were color coded if the genes were associated with response to temperature, turbulence, energy generation, or carbon metabolism. The numbers labeled inside the circles indicated the over-expressed genes: 1. chaperonin; 2. GTPases; 3. cytochrome c551/c552; 4. proteins of unknown function; 5. fimbrial protein; 6. NADH dehydrogenase; 7. rhodanese; 8. cold shock protein; 9. bacterial nucleoid DNA-binding protein; 10. isocitrate dehydrogenase; 11. polysulfide oxidoreductase; 12. truncated hemoglobins; 13. suncinyl-CoA synthetase; 14. succinate dehydrogenase; 15. desulfoferrodoxin; 16. ferredoxin; 17. cytochrome c; 18. F0F1-type ATP synthase; 19. pyruvate:ferredoxin oxidoreductase; 20. NADPH-dependent glutamate synthase; 21. acyl carrier protein; 22. translation initiation factor 1; 23. ABC-type phosphate transport system.

were collected in the present study. A conceptual model has been established that combines the geochemistry, hydrology, and multi-omics analyses of the *Sulfurihydrogenibium*-dominated filamentous microbial mats within the hot-spring drainage system (Fig. 9).

A suite of syntenous gene complexes (*e.g.,* heterodisulfide reductases, and other Fe-S reductases, including *hdr, sqr, rdh, sox, cox, psr/sre*) has been identified in both *Sulfurihydrogenibium* genomes and filamentous microbial mat metagenomes (Reysenbach *et al.,* 2009; Inskeep *et al.,* 2010; Takacs-Vesbach *et al.,* 2013). However, a pilot metatranscriptomic study showed expression of only one sulfide oxidation gene in the MHS streamer communities (Hamamura *et al.,* 2013). Based on metagenomic and metatranscriptomic studies, *Sulfurihydrogenibium* spp. also contain and express genes for arsenic cycling, including the potential to oxidize arsenite or reduce arsenate via ArsC and extrude arsenite via an efflux pump (AsrB) transformation to methylate arsenite via methyl transferases (Inskeep *et al.,* 2010; Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). The capacity to metabolize arsenic compounds has also been identified on *S. azorense* Az-Fu1 and *S. subterraneum* HGMK1 (Takai *et al.,* 2003; Aguiar *et al.,* 2004), which could use arsenite and arsenate as the electron donor and electron acceptor, respectively (Takai *et al.,* 2003). In addition, detection of a series of cytochromes (*e.g.,* cytochrome bd-I terminal oxidase, cbb_3 heme Cu oxidase) implies that *Sulfurihydrogenibium* spp. likely respires oxygen as the terminal electron acceptor (Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). The MHS *Sulfurihydrogenibium* spp. streamers experience As^{3+} concentration of about 20 μM, which is in the same order of magnitude as the sulfide concentration (100 μM) and implies that arsenite may be a significant energy source.

In addition, in order for the *Sulfurihydrogenibium*-dominated filamentous microbial community to survive the 70°C thermal stress, it must maintain cell integrity and enzymatic functions. Overexpressed transcripts and proteins associated with ''helper'' proteins and chaperonins would serve as adaptive physiological components that permit cell growth and replication (Figs. 7 and 8, Tables 1 and S11). Chaperonins facilitate appropriate folding of proteins at elevated temperature and prevent thermal denaturation by keeping them in a thermally stable configuration (Walter and Buchner, 2002).

5.2. rTCA cycle

Over-representation of the transcripts and the cognate proteins associated with the rTCA cycle support the interpretation of a chemoautotrophic lifestyle for the *Sulfurihydrogenibium* spp. in these CO_2-enriched ACF-depositing environments (Figs. 7–9, Tables 1 and S7). Here, trace amounts of dissolved organic carbon (~40 μM) limit

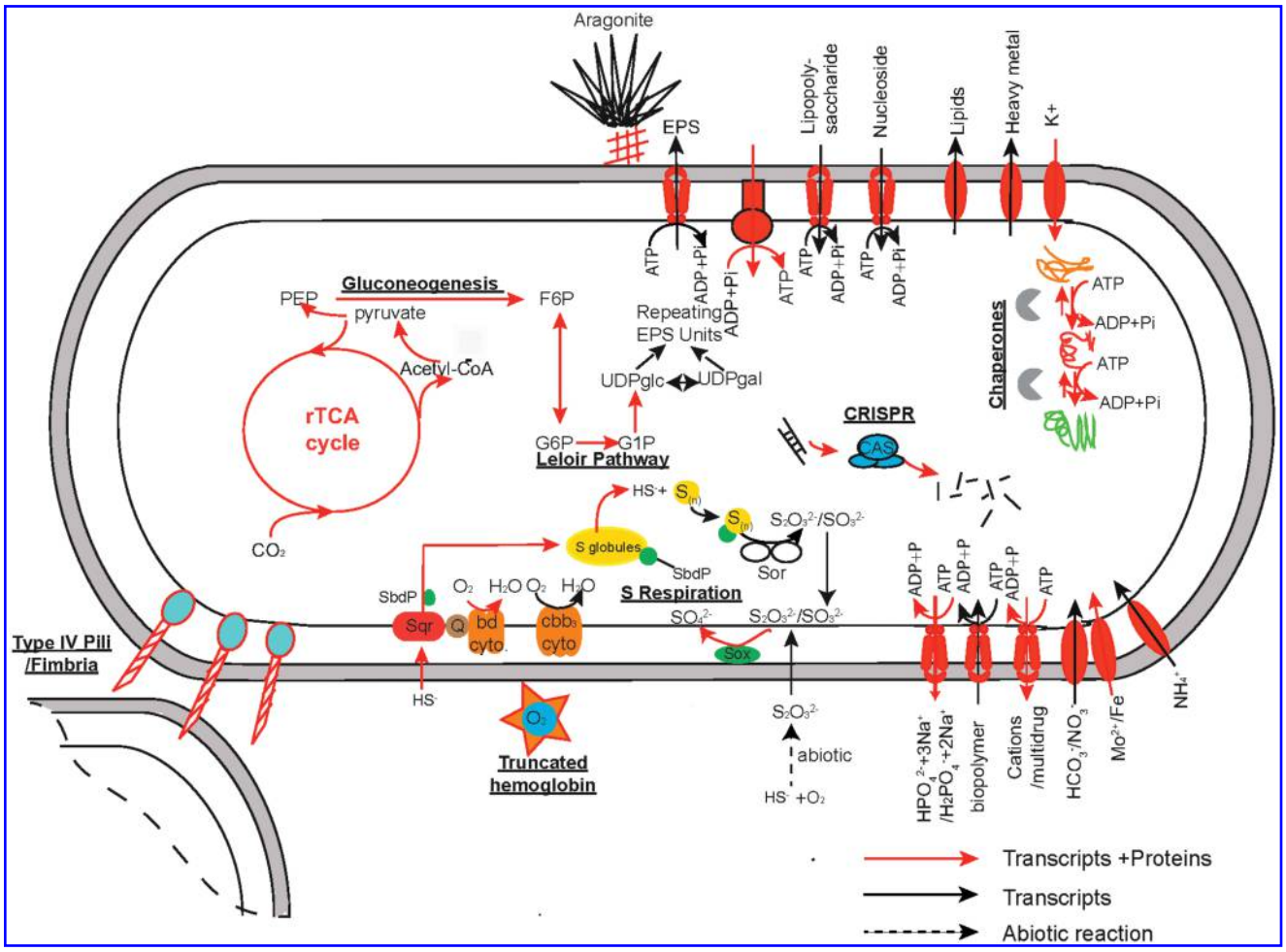

FIG. 9. Summary of reconstructed metabolic functions for the dominant *Sulfurihydrogenibium* spp. comprising the filamentous microbial mat community. Arrows connect successive steps of the metabolic pathways. Abbreviations: ATP: adenosine triphosphate; ADP: adenosine diphosphate; bd cyto: cytochrome bd-type quinol oxidase; CAS: CRISPR-associated genes; cbb3 cyto: cbb3-type cytochrome oxidase; F6P: fructose 6-phosphate; G6P: glucose 6-phosphate; G1P: glucose 1-phosphate; PEP: phosphoenolpyruvic acid; Q: quinone or ubiquinone; SbdP: sulfur-binding donating protein; $S_{(n)}$: zero valent sulfur; Sor: sulfur oxidation proteins; Sox: sulfite oxidase; Sqr: sulfide:quinone reductase; UDPglc: uridine diphosphate glucose; UDPgal: uridine diphosphate galactose.

heterotrophic metabolism. In addition, the temperature (70–73°C) is close to the upper limit for phototrophic metabolism (Brock, 1998). The rTCA cycle is an ancient mechanism for carbon fixation and is found in all the Aquificales organisms (*e.g., Sulfurihydrogenibium* spp.; Hugler *et al.,* 2007). This enables thermophilic microbes to take advantage of abundant products of dissolved CO_2 and its dissociation products and act as the primary producers in many hot-spring ecosystems (Reysenbach *et al.,* 2005; Inskeep *et al.,* 2010, 2013; Fouke, 2011; Takacs-Vesbach *et al.,* 2013). Presence of the rTCA cycle is further supported by the previous detection of phospholipid biomarkers and carbon isotopes in the same spring systems (Zhang *et al.,* 2004). In general, *Sulfurihydrogenibium* species are strict chemoautotrophs (Takai *et al.,* 2003; Aguiar *et al.,* 2004; Nakagawa *et al.,* 2005; Flores *et al.,* 2008; O'Neill *et al.,* 2008). Other examples include *Sulfurihydrogenibium*-dominated filamentous microbial communities from Coffee Pot Spring and the thermal vents in Yellowstone Lake that show biomarker genes affiliated with rTCA (*e.g., acl*A and *acl*B). This suggests that the rTCA cycle is a conserved biosynthesis trait for this group of organisms (Hall *et al.,* 2008; Yang *et al.,* 2011). Compared to the ACF at MHS, Calcite Spring water in YNP has significantly higher dissolved organic carbon but lower concentrations of dissolved inorganic carbon. Here *Sulfurihydrogenibium* and *Thermus* spp. co-occur in the streamer microbial community (Inskeep *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). In all of these locations, *Sulfurihydrogenibium* spp. does not dominate the filamentous microbial community.

5.3. Sulfur cycle

High concentrations of reduced sulfur compounds in ACF likely act as the primary energy source for the *Sulfurihydrogenibium* species (Figs. 7–9, Table S8). The functional genes, transcripts, and the cognate proteins associated with sulfide-quinone reductase (Sqr), sulfur oxidization proteins (SOX), and sulfide oxidase suggest *Sulfurihydrogenibium* spp. oxidizes a variety of reduced sulfur compounds

(*e.g.,* sulfide, S^0, thiosulfate, and sulfide). These predicted ecophysiological features are consistent to the known *Sulfurihydrogenibium* isolates (Aguiar *et al.,* 2004; Nakagawa *et al.,* 2005; Flores *et al.,* 2008; O'Neill *et al.,* 2008). Some sulfur compounds (*e.g.,* S^0) are thermodynamically stable. These microorganisms transfer sulfur globules to the appropriate enzymes to generate more soluble forms of polysulfide, which in turn are transformed by rhodanese and polysulfide reductase (Aussignargues *et al.,* 2012; Findlay, 2016) (Figs. 8 and 9; Table S8). *Sulfurihydrogenibium*-dominated filamentous mats found in hot springs around the world consistently contain reduced sulfur compounds (Reysenbach *et al.,* 2009; Inskeep *et al.,* 2010, 2013; Tamazawa *et al.,* 2012; Hamamura *et al.,* 2013; Takacs-Vesbach *et al.,* 2013). The addition of 5 mM thiosulfate to these mat communities enhances carbon fixation, and the oxidation of thiosulfate triggers energy production and biosynthesis (Yang *et al.,* 2011). This may explain why hot springs with relatively low concentrations of reduced sulfur are not dominated by *Sulfurihydrogenibium* spp. even though other geochemical factors (*e.g.,* pH, temperature, and dissolved oxygen) are comparable (Yang *et al.,* 2011; Hou *et al.,* 2013).

5.4. Aerobic respiration

The expression of a variety of aerobic respiration–associated genes may be what enables *Sulfurihydrogenibium* spp. to adapt and thrive in the dysoxic spring water of the ACF. Aerobic respiration by *Sulfurihydrogenibium* spp. in the dysoxic waters of ACF deposition (Fig. 1; Table S1) may be facilitated by some functional enzymes such as truncated hemoglobin-like proteins and cytochrome bd–type quinol oxidase (Figs. 8 and 9; Table S8). Hemoglobin superfamilies are associated with a variety of respiratory reactions (*e.g.,* NO dioxygenases or as NO detoxification components) under O_2-limiting conditions (Frey and Kallio, 2003; Gardner, 2000). Over-expressed truncated hemoglobin-like proteins are found in *Sulfurihydrogenibium* spp. and also observed in filamentous *Vitreoscilla* under hypoxic conditions, which are consistent with the low levels of terminal cytochrome oxidases seen in *E. coli* (Hardison, 1996). In addition, the uptake of oxygen in dysoxic conditions by *Sulfurihydrogenibium* spp. may also be assisted by cytochromes (de Gier *et al.,* 1996). These include cytochrome cbb3–type heme copper terminal oxidase and cytochrome bd–type quinol oxidase (Inskeep *et al.,* 2010; Takacs-Vesbach *et al.,* 2013). In addition, other alternative electron donating processes (*e.g.,* hydrogen oxidation and arsenic oxidation) are also utilized by *Sulfurihydrogenibium* spp. (Spear *et al.,* 2005; Hamamura *et al.,* 2013). However, meta-omic evidence suggests that neither of these processes is critical for the *Sulfurihydrogenibium* populations in the ACF depositional environment (Supplementary Information and Table S8).

5.5. Attachment of filamentous microbial mats

The combined attachment effects of EPSs, type IV pili, and surface stabilization via aragonite crystallization permit *Sulfurihydrogenibium* spp. to successfully colonize and outcompete other microbial communities in these hot-spring drainage environments (Figs. 1, 7, and 8). Successful colonization of the hot, shallow, and turbulent outflow environments of ACF fettuccini travertine deposition requires that *Sulfurihydrogenibium* spp. attach to the bottom surface of the spring drainage channel to form the streaming filamentous cell aggregates (Figs. 1–3). This is consistent with microbial streamers observed in many other natural and engineered systems, which are described as having a characteristic ''head'' attachment and ''tail'' downflow undulating filaments (Hallberg *et al.,* 2006; Niederberger *et al.,* 2009; Vrouwenvelder *et al.,* 2009; Ngene *et al.,* 2010). This allows them to withstand the strong hydrodynamic shear forces that sweep other planktonic and/or weakly adhered microorganisms downstream. Combined field, microscopy, and multi-omics evidence collected in the present study suggests *Sulfurihydrogenibium* spp. has an integrated strategy for attachment and growth that utilizes EPS, type IV pili, and rapid aragonite crystal precipitation (Fig. 8 and Tables 1, S9, and S10). EPS traps cells, water, ions, and other organic compounds on surfaces (Flemming *et al.,* 2000; Sutherland, 2001; Serra *et al.,* 2013). EPS also mediates the transition from reversible to irreversible adhesion of single cells and thus facilitates further attachment of motile bacteria (Stoodley *et al.,* 2002; Ducret *et al.,* 2012). Overexpression of type IV pili-associated transcripts and proteins in MHS *Sulfurihydrogenibium* spp. (Tables 1 and S10) suggests that they utilize their pili to securely attach (Takai *et al.,* 2003; Aguiar *et al.,* 2004; Nakagawa *et al.,* 2005; Flores *et al.,* 2008; O'Neill *et al.,* 2008). Unlike the swimming motility achieved by rotatory flagella, type IV pili facilitate colonization on hydrated organic or inorganic surfaces. Twitching motility is colonial in nature and usually involves cell-cell contact (Mattick, 2002).

Notably, flagella-associated transcripts are absent from the *Sulfurihydrogenibium* metatranscriptome at MHS but have been detected in filamentous microbial mats at Calcite Spring, YNP, and in the Azores (Hamamura *et al.,* 2013). This may at least in part be due to environmental differences in hydrodynamics and crystallization rate between these three sites. With respect to flow, filamentous microbial mats in Calcite Spring and the Azores have significantly slower hot-spring water flow rates ($\sim 0.1\ m\ s^{-1}$ in Calcite Springs, $\sim 0.05\ m\ s^{-1}$ in the Azores vs. $\sim 0.3\ m\ s^{-1}$ in MHS [Reysenbach *et al.,* 2005; Kandianis *et al.,* 2008; Takacs-Vesbach *et al.,* 2013]). Therefore, the flagella-associated genes in the lower-flow Calcite Springs and Azores systems may be expressed to allow organisms to be mobile and seek more optimal geochemical gradients. In contrast, the high flow rates in the ACF fettuccini streamer travertine depositing environments of MHS may prevent *Sulfurihydrogenibium* species from being equally motile (Hamamura *et al.,* 2013). Our *de novo* assembly suggests that the *Sulfurihydrogenibium* phylotypes at MHS do contain genes for type IV pili, which may act as alternative motility appendages (Table S6). With respect to mineralization, Calcite Spring and those in the Azores have very low rates of pyrite and S^0 mineral precipitation (Hamamura *et al.,* 2013), while travertine deposition at MHS is orders of magnitude higher at more than 5 mm per day (Fouke *et al.*, 2000; Fouke, 2011; Inskeep *et al.,* 2010, 2013; Takacs-Vesbach *et al.,* 2013). This extremely rapid encrustation may therefore limit or even prevent motility of the *Sulfurihydrogenibium*-dominated filamentous mats.

5.6. Fossilization of filamentous microbial mats

Apron and Channel Facies fettuccini streamer travertine deposits are an unequivocal microbial biomarker that faithfully preserves the morphology of the *Sulfurihydrogenibium*-

dominated microbial mats it entombs (Figs. 1–3). These deposits also create an environmentally sensitive and globally distributed fossil record of hot-spring water temperature, pH, and flux (Veysey *et al.,* 2008; Fouke, 2011; Okumura *et al.,* 2012; Capezzuoli *et al.,* 2014). Microbial metabolic processes that strongly influence ongoing travertine precipitation include the consumption and/or production of (1) CO_2 during oxidation-reduction coupled metabolic reactions and photosynthesis; (2) organic and inorganic acids; and (3) membrane-bound biomolecules and enzymes on outer cell walls (Kandianis *et al.,* 2008; Veysey *et al.,* 2008). These factors combine to control $CaCO_3$ precipitation rate, which in turn influences travertine mineralogy, chemistry, crystalline structure, porosity, and the preservation of other types of biomarkers such as biomolecules (*i.e.,* proteins, lipids, metabolites) and catabolic waste products (*i.e.,* isotopes, reduced electron acceptors).

Kandianis *et al.* (2008) demonstrated with controlled field experiments at MHS that the steady-state precipitation rate of ACF fettuccini streamer travertine was 2.5 times greater over a 3-day period at an aragonite saturation state (Ω_a) of 3.63 ± 0.09 (which is near that of normal seawater) than when microbial biomass is removed from the growing surface. This is likely caused by cell membrane–bound biomolecules and EPS concentrations in the microbial mat biomass (Kandianis *et al.,* 2008). This is consistent with previous studies that have shown proteins, polysaccharides, and lipids can alter $CaCO_3$ nucleation rates by changing the activation energy required for precipitation (Kandianis *et al.,* 2008; Jones and Peng, 2014; Cangemi *et al.,* 2016). One mechanism for this may be the negatively charged functional groups (*e.g.,* carboxyl, phosphate, and amine) on the microbial cell walls. These may either act as nucleation templates for adsorption of metal ions or create oversaturated crystal surface microenvironments for travertine precipitation (Warren *et al.,* 2001; Fein, 2006).

Alternatively, the EPS synthesized and excreted by microorganisms may also enhance travertine precipitation. Genes for the biosynthesis of protein precursors for EPS are present in all the *Sulfurihydrogenibium* genomes, suggesting it is a universal physiological feature for this group (Fig. 4 and Table S5). The corresponding transcripts and cognate proteins in the present study are also consistent with EPS production (Fig. 2 and Table S9). Because acidic residues and sugars in EPS may trap divalent cations (*e.g.,* Ca^{2+} and Mg^{2+}), partial degradation of EPS could generate locally high concentrations of Ca^{2+} and thus favor carbonate precipitation (Dupraz *et al.,* 2004). In addition, EPS can influence bacterial adhesion onto solid surfaces or improve the cohesion of sedimentary particles as during stromatolite (*microbialite*) formation (Tourney and Ngwenya, 2009, 2014). Modern travertine deposition in other hot springs has also observed that travertine preferentially nucleates on EPS, beginning with 5–80 nm diameter globules of amorphous calcium carbonates that coalesce and transform into well-developed acicular aragonite crystals (Greer *et al.,* 2017).

The fettuccini streamers and ridged morphology of ACF travertine are a direct result of the entombment of *Sulfurihydrogenibium*-dominated microbial mats by aragonite (Figs. 1–3). Each original microbial filament, up to 2 mm in diameter and 10 cm in length, is composed of smaller intertwined micron-scale microbial filaments and coccoidal cells (Figs. 1–3). Thick sheets of EPSs are draped between the filaments and contain abundant elliptical holes that are created by gas bubble release, gravity stretching, and water turbulence (Figs. 1–3; Fouke, 2011). These microbial filaments and EPS sheets become rapidly encrusted by small (<5 μm long), densely packed aragonite needles. Significantly larger (>>5 μm long) aragonite needles grow in the EPS-rich interstitial void spaces (Fig. 3C). Larger acicular aragonite crystals eventually encrust each filament and the interstitial EPS (Fig. 3D, 3E).

Upstream-to-downstream distributions of microbial phospholipid fatty acids and glycolipid fatty acids (Zhang *et al.,* 2004) parallel the travertine facies-specific microbial community partitioning indicated by 16S rRNA gene sequence libraries (Fouke *et al.,* 2003; Martin *et al.,* 2010). These biomolecules are entombed and preserved within the solid-phase streamer travertine, creating an invaluable additional biomarker to travertine morphology, crystalline fabric, and growth rate. Enrichment of fatty acid ^{13}C further supports the omics analyses in the present study, suggesting that these *Sulfurihydrogenibium*-dominated microbial communities are using the rTCA cycle (Zhang *et al.,* 2004). Conversely, sulfur-isotope fractionation due to microbial processes is not readily preserved in the ACF fettuccini streamer travertine (Fouke *et al.,* 2000). Microbial-driven sulfide oxidation in these environments is insufficient to overprint the large dissolved sulfur reservoir of the open spring system.

Field and microscope observations indicate that the *Sulfurihydrogenibium*-dominated microbial mats preferentially build up ridges of travertine on the bottom of the spring channels by combining the above-described suite of physiological and metabolic characteristics (Figs. 1–3). These run parallel to the downstream water flow direction, with the top of each ridge just millimeters below the flowing water surface. Clumps of living filaments are anchored solely on these ridges but not in the lows between ridges. Interpretations from the metagenomic, metatranscriptomic, and metaproteomic analyses suggest that these elevated travertine ridges maximize their access to miniscule amounts of dissolved oxygen, optimize their ability to attach and form large filaments that undulate in the down-current, and allow *Sulfurihydrogenibium* spp. to thrive in these hot-spring outflow channels at the virtual exclusion of other microorganisms.

6. Conclusions

Filamentous microbial mats that are 87–98% dominated by the bacterium *Sulfurihydrogenibium* spp. thrive in high-temperature fast-flowing turbulent water with steep redox gradients at MHS in YNP. Hot-spring travertine rapidly entombs these filamentous mats to form fettuccini-like travertine streamer microbial biomarkers that are important targets in the search for ancient fossilized microbial life on Earth and potentially other planets. Field observations integrated with geology, hydrology, microscopy, and multi-omic molecular biology analyses indicate that multiple *Sulfurihydrogenibium* spp. physiological and metabolic adaptations have allowed them to optimize their lifestyles in these hot-spring drainage environments to the virtual exclusion of other microorganisms. The formation of pili and EPS production allow for physical attachment and stability in these high-shear turbulent flow regimes. In addition, they combine the oxidation of reduced sulfur (*e.g.,* sulfide, sulfur, or thiosulfate) with a simultaneous requirement for the uptake of

extremely low concentrations of dissolved O_2. This results in the deposition of centimeter-scale elevated travertine streamer ridges that allow *Sulfurihydrogenibium* spp. to access low levels of dissolved oxygen while optimizing their ability to anchor in turbulent outflow channels at the virtual exclusion of other microorganisms. These physiological and metabolic attributes of *Sulfurihydrogenibium*-dominated filamentous mat communities can be directly inferred and reconstructed from fossilized ridged travertine streamer microbial biomarker deposits.

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Abbreviations Used

ACF = Apron and Channel Facies
COGs = clusters of orthologous groups of proteins
EPSs = extracellular polymeric substances
MHS = Mammoth Hot Springs
ORFs = open reading frames
rTCA cycle = reversed tricarboxylic acid cycle
SDS = sodium dodecyl sulfate
SOX = sulfur oxidization proteins
Sqr = sulfide-quinone reductase
SRA = Short Reads Archive
YNP = Yellowstone National Park