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Jay, Z
Rusch, D
Tringe, Susannah
et al.

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Predominant *Acidilobus*-Like Populations from Geothermal Environments in Yellowstone National Park Exhibit Similar Metabolic Potential in Different Hypoxic Microbial Communities

Z. J. Jay,[a] D. B. Rusch,[b] S. G. Tringe,[c] C. Bailey,[a] R. M. Jennings,[a] W. P. Inskeep[a]

Thermal Biology Institute and Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana, USA[a]; Center for Genomics and Bioinformatics, Indiana University, Bloomington, Indiana, USA[b]; Department of Energy-Joint Genome Institute, Walnut Creek, California, USA[c]

High-temperature (>70°C) ecosystems in Yellowstone National Park (YNP) provide an unparalleled opportunity to study chemotrophic archaea and their role in microbial community structure and function under highly constrained geochemical conditions. *Acidilobus* spp. (order *Desulfurococcales*) comprise one of the dominant phylotypes in hypoxic geothermal sulfur sediment and Fe(III)-oxide environments along with members of the *Thermoproteales* and *Sulfolobales*. Consequently, the primary goals of the current study were to analyze and compare replicate *de novo* sequence assemblies of *Acidilobus*-like populations from four different mildly acidic (pH 3.3 to 6.1) high-temperature (72°C to 82°C) environments and to identify metabolic pathways and/or protein-encoding genes that provide a detailed foundation of the potential functional role of these populations *in situ*. *De novo* assemblies of the highly similar *Acidilobus*-like populations (>99% 16S rRNA gene identity) represent near-complete consensus genomes based on an inventory of single-copy genes, deduced metabolic potential, and assembly statistics generated across sites. Functional analysis of coding sequences and confirmation of gene transcription by *Acidilobus*-like populations provide evidence that they are primarily chemoorganoheterotrophs, generating acetyl coenzyme A (acetyl-CoA) via the degradation of carbohydrates, lipids, and proteins, and auxotrophic with respect to several external vitamins, cofactors, and metabolites. No obvious pathways or protein-encoding genes responsible for the dissimilatory reduction of sulfur were identified. The presence of a formate dehydrogenase (Fdh) and other protein-encoding genes involved in mixed-acid fermentation supports the hypothesis that *Acidilobus* spp. function as degraders of complex organic constituents in high-temperature, mildly acidic, hypoxic geothermal systems.

Microbial communities in high-temperature geothermal environments provide unique opportunities for determining the physiology of specific populations and for studying geobiological interactions central to a comprehensive functional and evolutionary understanding of thermophilic microorganisms. Acidic, hyperthermal (>70°C) systems from Yellowstone National Park (YNP) contain significant concentrations of reduced constituents such as H_2, H_2S, elemental sulfur, As(III) (e.g., H_3AsO_3), Fe(II), and organic carbon (1, 2), which are often used as electron donors for different chemotrophic microorganisms (3). The oxidation of reduced compounds is coupled with the reduction of terminal electron acceptors, including O_2, Fe(III), and different sulfur species, generating energy for cellular processes (3, 4). Thermophilic organisms catalyze reactions of geochemical significance (e.g., oxidation-reduction, biomineralization, acidification, and mineral dissolution) that are often orders of magnitude faster than the corresponding abiotic mechanisms (4–7).

High-temperature acidic geothermal systems contain several predominant archaeal populations within the phylum *Crenarchaeota* (i.e., organisms within the orders *Desulfurococcales*, *Thermoproteales*, and *Sulfolobales*). Nearly all cultivated representatives of this phylum can generate energy through the oxidation and/or fermentation of complex organic compounds (e.g., yeast extract or peptone), and this is often coupled with the reduction of elemental sulfur (8–10). With the exception of *Acidilobus saccharovorans* (11), other sequenced *Desulfurococcales* are too unrelated to those present in YNP to be of significant value for an understanding of community function (i.e., *Aeropyrum pernix* K1 [12], *Desulfurococcus kamchatkensis* [13], *Hyperthermus butylicus* [14], *Ignicoccus hospitalis* [15], and *Staphylothermus marinus* [16]). However, misannotation of specific genes within *Ac. saccharovorans* has led to misinterpretation regarding the functional potential of *Acidilobus* spp. Furthermore, previous suggestions that *Caldisphaera* and *Acidilobus* spp. comprised a new order (*Acidilobales*) within the class *Thermoprotei* (*Crenarchaeota*) (8, 11, 17) have been clarified, and phylogenetic analyses indicate that these organisms belong within the *Desulfurococcales* (18). Common genomic and physiological attributes of sequenced members of the *Desulfurococcales* include pathways for catabolism and anabolism of complex carbon sources, including both proteins and carbohydrates. *Pyrolobus fumarii* and *I. hospitalis* are the only obligate autotrophs identified within this group and fix carbon dioxide via the dicarboxylase/4-hydroxybutyrate (DC/HB) cycle using pyruvate synthase and phosphoenolpyruvate (PEP) carboxylase (19).

Organisms within the *Desulfurococcales* are abundant community members in high-temperature habitats of YNP, especially

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Address correspondence to W. P. Inskeep, binskeep@montana.edu.
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Acidilobus-like populations found in mildly acidic hypoxic environments containing elemental sulfur (4, 20, 21). Similar organisms are found in numerous other high-temperature sulfide-rich systems distributed globally (22–28). The presence of nearly identical 16S rRNA gene sequences (>99% nucleotide identity) in Fe-oxide mats containing little to no elemental sulfur prompted questions regarding the potential variation in physiological attributes of these organisms across habitats with different geochemical properties (29). Members of the *Desulfurococcales* generally lack heme Cu oxidases (HCOs) required for aerobic respiration, and although *Aeropyrum pernix* is a notable exception (30), the majority of cultured *Desulfurococcales* are considered anaerobic and contain at least one of the subunits of the cytochrome *bd* ubiquinol oxidases thought to be important for aerobic respiration under low-oxygen conditions or in scavenging oxygen (31). Consequently, knowledge of the physiological potential of members of the *Desulfurococcales* provides information critical for microbial community analysis and modeling as well as evolutionary studies focused on the role of archaea in early Earth (e.g., prior to the great oxidation event, 2.4 billion years [Ga] [32–34]).

The primary aim of the current study was to determine the functional role of *Acidilobus*-like populations from four different high-temperature environments of YNP across a range of pH values and dissolved oxygen (DO)/dissolved sulfide (DS) concentrations. A recent metagenome study of 20 geothermal sites (35) included several systems where *Acidilobus*-like populations ranged in abundance from ~25 to 70% of the microbial community, based on an analysis of individual sequence reads and metagenome assemblies (29). The importance of *Acidilobus*-like organisms across broad pH ranges and habitat types (Fe versus S) prompted further and more detailed genomic analyses coupled with additional experimental evidence to determine their potential role *in situ*. The specific objectives of this study were to (i) conduct detailed comparative genomics and metabolic reconstruction of *Desulfurococcales* populations identified in four distinct geothermal habitats ranging in pH from 3 to 6, (ii) identify candidate protein-encoding genes of the YNP *Desulfurococcales* populations that may be responsible for key geochemical processes and microbial community interactions in these environments, and (iii) determine the activity of these populations *in situ* using reverse transcriptase PCR (RT-PCR) of genes important for phylogenetic and functional analysis. Our results demonstrate that the dominant *Desulfurococcales* populations found across different high-temperature habitats (pH 3 to 6) are highly similar and share common metabolic attributes across sites. Candidate proteins associated with the degradation of complex carbon, fermentation, and low-oxygen conditions are important in defining the functional role of these organisms in hypoxic high-temperature environments.

MATERIALS AND METHODS

Geothermal sites. The sites discussed in the current study were included in a larger metagenome study (Community Sequencing Project [CSP] 787081) that identified *Acidilobus*-like populations as important community members of mildly acidic (pH 3.3 to 6.1), high-temperature (>70°C) geothermal systems (29, 35). Briefly, four different geothermal microbial communities (Fig. 1) were sampled from August to November 2007 at the following locations: Norris Geyser Basin One Hundred Spring Plain (OSP), Monarch Geyser (MG), Cistern Spring (CIS), and Joseph's Coat Hot Springs-Scorodite Spring (JCHS). All sites have been characterized extensively for several years, and the geochemistry is reasonably stable in

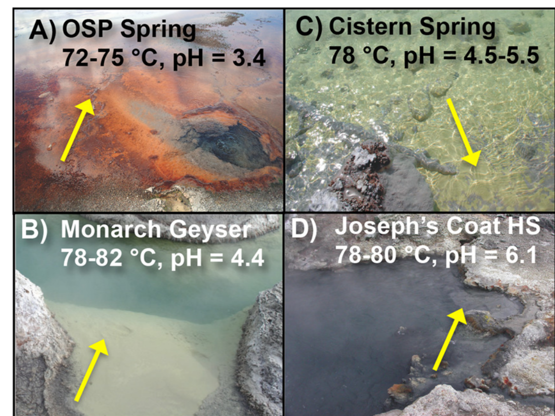

FIG 1 Four high-temperature chemotrophic systems containing either iron oxide mats or sulfidic sediments were sampled for metagenome sequencing and geochemical analysis. (A) One Hundred Spring Plain (OSP); (B) Monarch Geyser (MG); (C) Cistern Spring (CIS); (D) Joseph's Coat Hot Springs (JCHS). Yellow arrows indicate sampling locations. Site geochemistry is listed in Table S1 in the supplemental material.

these systems (2, 4, 6, 7, 21, 36). Subsequent samples from identical locations and with identical temperature values were obtained in 2009 and 2010 for reverse transcriptase assays as well as continued geochemical monitoring. The location and geochemical and physical characteristics of each sampling site (see Table S1 in the supplemental material) are critical to an understanding of the interplay between indigenous microbial populations and geochemical processes. Mat samples taken from the OSP spring were located in the primary outflow channel 2 to 3 m down the gradient from the source pool (82°C, pH 3.3, and ~1.5-m diameter) at a temperature of 72°C to 75°C and consist of amorphous Fe-oxyhydroxides [0.6 mol As(V)/mol Fe (7)]. MG, CIS, and JCHS are all hypoxic systems, which contain dissolved sulfide (DS) and elemental sulfur sediments (21). All sediment samples were collected aseptically into 50-ml falcon tubes, placed on dry ice, and then stored at −80°C until DNA was extracted for metagenomic sequencing and/or PCR amplification. Sediment samples collected for RNA extraction were mixed (1:5, vol/vol) with RNAlater (Life Technologies, Grand Island, NY, USA), kept at ~4°C overnight, and then stored at −80°C until extraction.

Aqueous geochemistry. Spring water geochemistry was analyzed according to protocols described previously (4, 6, 21). Dissolved oxygen (DO) and DS concentrations were determined on-site using a closed-headspace modification of the Winkler protocol and the amine sulfuric acid method, respectively (21, 37). Dissolved gasses (CO_2, H_2, and CH_4) were analyzed by utilizing headspace gas chromatography (GC) as described previously (4). Briefly, closed-headspace samples of filtered (0.2-μm pore size) spring water were collected into 160-ml glass serum bottles by use of a peristaltic pump and stored with zero headspace with butyl stoppers. A known volume of liquid was withdrawn and replaced with an equivalent volume of atmosphere. After equilibration with gentle shaking for 60 min at room temperature, headspace samples were injected into a Varian gas chromatograph (model CP2900) using Ar and N_2 as carrier gases and analyzed on a dual-channel thermal conductivity detector system. Headspace gas concentrations were employed to calculate original dissolved gas concentrations using Henry's law constants (adjusted to equilibration temperatures that ranged from 20°C to 25°C) for each gas (3) and a mass balance equation for total dissolved gas prior to headspace equilibration. Two aqueous samples at each location were filtered (0.2-μm pore size) on-site into sterile 50-ml screw-cap tubes. One sample was preserved with ultrapure 0.1 M HNO_3 and then analyzed using inductively coupled plasma spectrometry (ICP) for total dissolved Ca, Mg, Na, K, Si, Al, As, Fe, B, and trace elements, including Cd, Cr, Cu, Mn, Ni, Pb, Sb, Se, and Zn. The unacidified sample was analyzed for F^-,

Cl^-, SO_4^{2-}, NO_3^-, $S_2O_3^{2-}$, and AsO_4^{3-} using ion-exchange chromatography (IC) (AS16 4-mm column; Dionex Corp., Sunnyvale, CA, USA). Levels of dissolved inorganic C (DIC), dissolved organic C (DOC), and total dissolved nitrogen were determined on separate closed-headspace, filtered samples by use of a total organic/inorganic carbon and nitrogen analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA).

Nucleic acid extraction. Extraction of total DNA for metagenome sequencing was performed using the PowerMax Soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) for MG, CIS, and JCHS sediments or using the FastDNA Spin kit for soil (MP Biomedicals, Santa Ana, CA, USA) for OSP (sites 3, 19, 4, and 8 of the Department of Energy-Joint Genome Institute [DOE-JGI] CSP, respectively). The amount of DNA extracted from sulfur sediments was smaller than that obtained from Fe-oxide mats (~0.2 to 0.35 μg DNA/g wet sediment in S systems versus ~1.0 μg/g in Fe mats from OSP). Total RNA was extracted using either the FastRNA Pro Soil-Direct kit (MP Biomedicals, Santa Ana, CA, USA) or Tri reagent (RNA isolation reagent) (Sigma-Aldrich, St. Louis, MO, USA). Briefly, 1-ml aliquots of RNAlater-preserved sediments sampled in 2009 to 2010 were lysed using three alternating freeze [N_2(l)]-thaw (40°C heat block) cycles and then extracted by mixing 1 to 2 ml Tri reagent and incubating the mixture at room temperature for 5 min before the addition of 200 to 400 μl chloroform. The mixture was allowed to sit for 15 min before centrifugation at 13,000 rpm for 15 min at 4°C. The top aqueous phase was removed, and the RNA was precipitated by addition of 500 μl ice-cold 100% isopropanol per 1 ml Tri reagent and stored at −20°C overnight. The precipitated RNA was pelleted (13,000 rpm for 15 min at 4°C), washed with 750 μl ice-cold 70% ethanol, air dried, then resuspended in a solution containing 90 μl RNase-free water, 10 μl DNase I buffer, and 2 μl (4 U) DNase I (New England BioLabs, Ipswich, MA, USA) and incubated at 37°C for 40 min. The DNA-free RNA was reprecipitated with 20 μl 8 M LiCl and 250 μl ice-cold 100% ethanol and placed at −20°C overnight (38). The RNA was pelleted by centrifugation at 13,000 rpm for 15 min at 4°C and then washed with 500 μl ice-cold 70% ethanol before a final centrifugation (15 min at 13,000 rpm and 4°C). The pellet was air dried at room temperature and resuspended in either 50 μl RNase-free water or a 10:1 dilution of Tris-EDTA (TE) buffer (pH 7) (Life Technologies, Grand Island, NY, USA). Quantification and purity were estimated with a NanoDrop UV-visible (UV-Vis) spectrophotometer (NanoDrop, Wilmington, DE, USA).

Metagenome sequencing, assembly, and annotation. Random shotgun sequencing was performed using paired-end libraries (~3 kb) constructed from environmental DNA extracts (described above). Sequencing (Sanger and 454 platforms) was conducted at the DOE-JGI, Walnut Creek, CA, as part of a larger effort to characterize thermophilic microbial communities across different habitat types (35) (Community Sequencing Project 787081). The assembly of individual sequences (Sanger and 454 read lengths averaged 800 and 350 bp, respectively) into larger contigs and scaffolds was performed using Celera Assembler (version 4.0), as described previously (35, 39), with the following parameters: doOverlapTrimming, 0; doFragmentCorrection, 0; globalErrorRate, 12; utgErrorRate, 150; utgBubblePopping, 1; useBogUnitig, 0. The *Acidilobus*-like sequence in each sample was extracted from the assembled metagenome sequence by use of nucleotide word frequency–principal-components analysis (NWF-PCA) (39, 40) (http://gos.jcvi.org/openAccess/scatterPlotViewer.html) and then manually curated for purity by screening the percent G+C content of each scaffold, NCBI-BLAST comparisons to published genomes, whole-genome alignments, and detailed phylogenetic analysis of numerous single-copy genes. Automated annotation of the assembled sequence was conducted using DOE-JGI Integrated Microbial Genomes and Metagenomes Expert Review (IMG-MER and IMG-ER) (http://img.jgi.doe.gov/) (41) and RAST (Rapid Annotation Using Subsystem Technology) (42). The protein-encoding sequence from the *de novo* assembly (site MG) was subjected to detailed analyses of pathway and genome completeness, which included gene neighborhood analysis, amino acid alignments to proteins with crystal structures, and phylogenetic analysis. As discussed below, the *de novo* assemblies are highly similar to one another, and the primary reason to emphasize the MG assembly is that the assembly is represented by significantly higher coverage than other sites receiving only Sanger sequencing. Approximately 30% of the coding sequences (CDSs) in the *Acidilobus* assemblies were annotated as hypothetical genes or genes of unknown function, which is average for current archaeal genomes.

Phylogenetics and comparative genomics. All sequence alignments (i.e., 16S rRNA genes, 60 single-copy proteins, and metabolic proteins) were initially generated using ClustalW and/or MUSCLE (43), manually edited, and then used to construct phylogenetic trees by employing neighbor-joining and/or maximum likelihood methods (MEGA 5.0) (bootstrap values were determined with either 100 or 1,000 resamplings) (44). Sixty single-copy transcription and translational proteins shared across numerous *Archaea* (see Table S2 in the supplemental material) were concatenated prior to phylogenetic analysis (34, 45–48).

The genome sequence of the *Acidilobus*-like populations was compared to other published *Desulfurococcales* genome sequences by use of various utilities, including NWF-PCA (40, 49), NCBI-BLAST algorithms, IMG-ER tools, MAUVE 2 (50, 51), ACT (Artemis Comparison Tool) (52), and Seed Viewer (http://rast.nmpdr.org/seedviewer.cgi). NWF-PCA plots were generated with the JCVI upload utility scatter plot viewer (http://gos.jcvi.org/openAccess/uploadScatterplotForm3.html) with the following parameters: word size of 4, normalized for GC content, and 10,000-bp chop size with 100-bp overlap. Protein homologs shared between the *Acidilobus*-like *de novo* assemblies, *Ac. saccharovorans*, and *Ae. pernix* were identified using BLASTP homology cutoffs (≥30% amino acid identity and ≥70% protein length), then compared using a Venn diagram (http://omics.pnl.gov/software/VennDiagramPlotter.php).

PCR and RT-PCR. Multiple primer sets were designed (primer-BLAST [http://www.ncbi.nlm.nih.gov/tools/primer-blast/] and Integrated DNA Technologies [IDT] OligoAnalyzer 3.1) to target specific *Acidilobus*-like phylogenetic and functional marker genes (see Table S3 in the supplemental material) *in situ*. All primer sets were synthesized by IDT (Coralville, IA) and used for PCR amplification of environmental DNA under the following conditions (GoTaq Green Master Mix [Promega Corp., Madison, WI, USA]): 95°C for 5 min; 30 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 2 min; and a final extension step of 72°C for 5 min. PCR products were sequenced at the Plant-Microbe Genomics Facility at Ohio State University (Columbus, OH, USA) to verify product sequence. Reverse transcriptase PCR (RT-PCR) reactions of environmental RNA were performed with the Access RT-PCR system (Promega Corp., Madison, WI, USA) according to the product instructions but with the following reaction conditions: 45°C for 45 min; 95°C for 5 min; 40 cycles of 95°C for 45 s, 55°C for 45 s, and 70°C for 45 s; and 70°C for 5 min. For every RT-PCR, samples were amplified in triplicate, and three control reactions were included (i) to test nonspecific amplification (no-template control), (ii) to check for DNA contamination (exclusion of the reverse transcriptase), and (iii) as a positive control (DNA instead of RNA template). All PCR and RT-PCR products were assessed with a 1% agarose gel and stained with ethidium bromide.

Accession numbers. NCBI GenBank accession numbers for the four *de novo* assemblies are available under the registered Bioproject PRJNA221622.

RESULTS

Phylogenetic analysis and genome properties of *de novo Acidilobus* assemblies. Complete sets of single-copy genes were identified in all four *Acidilobus*-like *de novo* assemblies, including 5S, 16S, and 23S rRNA genes as well as genes involved in replication, transcription, translation, and numerous biosynthetic pathways (see Tables S2 and S4 in the supplemental material). Phylogenetic analysis of the assembled genome sequence was conducted using 16S rRNA gene sequences (Fig. 2A) as well as 60 concatenated, single-copy proteins conserved across the domain *Archaea* (Fig. 2B). The environmental *Desulfurococcales*-like populations from

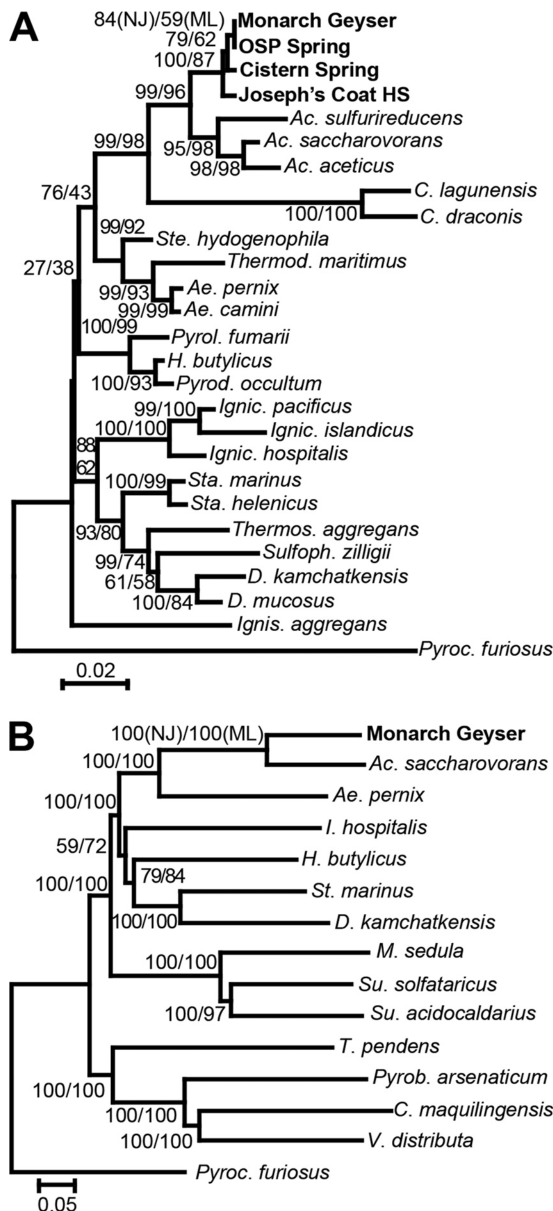

FIG 2 Phylogenetic analysis of 16S rRNA gene sequences (A) and 60 concatenated, single-copy transcriptional/translational proteins (B) identified in the *Acidilobus*-like *de novo* assemblies compared to other members of the phylum *Crenarchaeota*. Trees were constructed using neighbor-joining (NJ) and maximum likelihood (ML) analyses; bootstrap values were determined by resampling 1,000 (neighbor joining) or 100 (maximum likelihood) replicate trees. The scale bars represent substitutions per 100 positions. *Pyrococcus furiosus* (phylum *Euryarchaeota*) was used as the outgroup in both analyses.

YNP group consistently with *Ac. saccharovorans*, which supports the phylogenetic placement of all four populations into the genus *Acidilobus* (order *Desulfurococcales*). Nearly identical full-length 16S rRNA gene sequences have been observed in these same sites upon repetitive sampling (4), which indicates a consistent presence of this specific phylotype in acidic (pH 3 to 6), thermal (70°C to 80°C) habitats of YNP. Related *Acidilobus* populations are also observed in sulfidic thermal habitats in Kamchatka and Japan (28, 53); however, the 16S rRNA gene sequences of the YNP populations are only 97% identical to either *Acidilobus sulfurireducens* or *Ac. saccharovorans*, which were isolated from Yellowstone National Park (54) and Kamchatka, Russia (17), respectively. Other *Desulfurococcales* sequences observed in these sites include relatives of *Caldisphaera* spp. (21, 55); however, these were not observed as abundant populations (<1%) in the four sites studied here.

Each *Acidilobus* assembly contained between ~1.4 and 1.5 Mbp of nonredundant sequence, which is similar to the genome sizes observed for other members of the *Desulfurococcales* (Table 1). The average contig/scaffold coverage for Sanger assemblies ranged from 5 to 10× across these four sites, and higher coverage (~68×) was obtained from the sulfur sediments at Monarch Geyser (MG) using 454 pyrosequencing. The *Acidilobus*-like populations represented ~25 to 70% of the sequence reads observed in these microbial communities (29) (see Fig. S1 in the supplemental material). The average G+C content (percent) of each assembly was nearly identical, ranging from 59.1 to 59.3%, which is higher than that observed for *Ac. saccharovorans* (57%) (11) or *Ac. sulfurireducens* (56%) (39). A smaller set of large scaffolds (<10) contained the majority of nonredundant assembled sequence; for example, the largest scaffolds observed in OSP, MG, CIS, and JCHS were 0.38, 0.47, 0.35, and 0.39 Mb, respectively. Gene annotation and curation identified between 1,634 and 1,789 coding sequences (CDSs) in the JCHS and MG assemblies, and at least one tRNA for all 20 amino acids was identified in each sample (the only exception was a missing histidine tRNA gene in the CIS assembly [see Table S5 in the supplemental material]). No CRISPR/Cas elements were identified in any of the consensus assemblies (56). Although repetitive elements may assemble poorly (57), the average read length of both Sanger and 454 sequencing platforms and the extensive coverage for MG assemblies (~68×) should have been sufficient for detecting these sequences. Consequently, the genomic data suggest that CRISPR/Cas proteins present in other archaea are not observed in this phylotype.

Nucleotide word frequency–principal-components analysis (39, 40) of assembled *Acidilobus*-like sequence from each site reveals the strong similarity in sequence character (i.e., G+C content and codon usage bias) among the four assemblies as well as similarity to the full-genome sequence of *Ac. saccharovorans* (Fig. 3A). Genome comparisons among the four assemblies show that a large majority (82 to 85%) of deduced sequence is highly identical (>90% amino acid identity) and that the genomes are highly syntenous to one another (e.g., see Fig. S4 and S7 in the supplemental material). Other reference organisms within the *Desulfurococcales* (e.g., *I. hospitalis*, *Thermosphaera aggregans*, and *Ae. pernix*) exhibit significantly different sequence character than either *Ac. saccharovorans* or the *Acidilobus*-like assemblies from YNP (Fig. 3A). BLASTP comparisons (≥30% amino acid identity and ≥70% protein length) among annotated CDSs of *Ac. saccharovorans*, *Ae. pernix*, and the *Acidilobus*-like assembly from MG revealed 478 CDSs conserved across all three organisms (Fig. 3B). However, the MG assembly shared only 574 CDSs (32%) with *Ae. pernix*, compared to 1,412 (79%) with *Ac. saccharovorans*. A majority of CDSs (64%; 1,082) in *Ae. pernix* did not have homologs in either *Ac. saccharovorans* or the *de novo* assemblies from YNP (the assembly for MG is shown, but results are within ±3 to 8% for other YNP assemblies).

***In situ* gene expression.** The activity and function of the YNP *Acidilobus* populations were evaluated using primers for a fairly extensive set ($n = 13$) of conserved phylogenetic genes and can-

TABLE 1 Genome sequence and habitat characteristics of *de novo Acidilobus*-like assemblies from Yellowstone National Park compared to reference isolates[a] within the order *Desulfurococcales* (phylum *Crenarchaeota*)[g]

Site location or isolate	Temp (°C)	pH	kbp (no. of scaffolds)	G+C content (%)	No. of genes	No. of CDSs	No. of RNAs	% coding	Location
Sites									
OSP	72–75	3.3	1,431 (42)	59.1	1,745	1,697	48	90	NGB, YNP, USA
Monarch Geyser	78–82	4	1,545 (35)	59.1	1,841	1,789	52	91	NGB, YNP, USA
Cistern Spring	78–82	4.5–5.5	1,423 (18)	59.3	1,721	1,673	48	90	NGB, YNP, USA
Joseph's Coat Hot Spring	78–82	6.1	1,477 (13)	59.2	1,683	1,634	49	91	JCTA, YNP, USA
Isolates									
Ac. saccharovorans 345-15[b]	60–90	2.5–5.8 (3.5–4)	1,496	57.2	1,551	1,499	48	90	Uzon caldera, Kamchatka, Russia
Ae. pernix K1[c]	80–85	7	1,670	56.1	2,439	2,386	53	89	Marine, Kodakara Island, Japan
H. butylicus DSM 5456[d]	90–98	7	1,667	53.6	1,671	1,607	52	81	Marine, S. Miguel, Açores
I. hospitalis KIN4/I[e]	95	4.5–7	1,298	56.5	1,494	1,441	51	93	Marine, Kolbeinsey Ridge, Iceland
D. kamchatkensis 1221n[f]	80–108	6.5	1,365	45	1,523	1,472	50	88	Uzon caldera, Kamchatka, Russia

[a] For isolates, the temperature and pH optima are listed.
[b] See references 11 and 17.
[c] See references 12 and 30.
[d] See references 14 and 90.
[e] See references 15, 91, and 92.
[f] See references 13 and 26.
[g] CDS, coding sequences; NGB, Norris Geyser Basin; JCTA, Joseph's Coat Thermal Area.

didate functional genes (see Table S3 in the supplemental material). 16S rRNA was successfully amplified from RNA extracted from all four geothermal locations (OSP, MG, CIS, and JCHS), indicating activity of the indigenous *Acidilobus*-like populations *in situ*. Other transcripts detected in RNA extracted from MG included RNA polymerase subunit B (*rpoB*), formate dehydrogenase subunit H (*fdhF*), glutamate synthase (*gltD*), quinol oxidases *cydA* and *cydA′*, amino-acid-catabolizing pyruvate:ferrodoxin oxidoreductase (POR) and 2-ketoisovalerate:ferridoxin oxidoreductase (VOR) enzymes, and lactate dehydrogenase. These results show that the predominant *Acidilobus*-like populations identified using metagenome sequencing are metabolically active and that key genes involved in central C metabolism, fermentation, and respiration are expressed in sulfur sediment microbial communities. Further analyses of the metabolic attributes of this phylotype provide context for the importance of these functional genes in defining the realized niche of these organisms in Fe-oxide and sulfur sediment communities.

Metabolic potential of *Acidilobus*-like populations from YNP. (i) Central carbon metabolism. To determine the likely function of indigenous *Acidilobus*-like populations across disparate habitat types, we performed metabolic reconstruction using annotated and manually curated *de novo* assemblies. Genes coding for multiple proteases, peptidases, hydrolases, aminotransferases, and dipeptide/oligopeptide ABC transporters were identified in each assembly. CDS regions implicated in carbohydrate uptake,

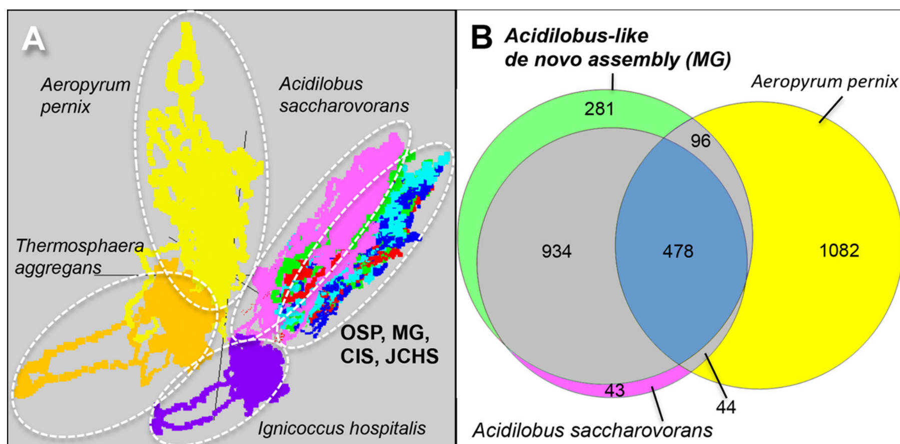

FIG 3 (A) Nucleotide word frequency–principal-components analysis of four *Acidilobus*-like sequence assemblies compared to other reference genomes. For *de novo* assemblies, One Hundred Spring Plain (OSP) is in red; Monarch Geyser (MG) is in green, Cistern Spring (CIS) is in sky blue, and Joseph's Coat Hot Springs (JCHS) is in dark blue. For reference organisms, *Acidilobus saccharovorans* is in pink, *Ignicoccus hospitalis* is in violet, *Thermosphaera aggregans* is in gold, and *Aeropyrum pernix* is in yellow. (B) Venn diagram comparing similarity (>30% amino acid identity and >70% protein length) of coding genes between the *Acidilobus*-like assembly from site MG (green) (1,789 total coding sequences) and the genomes of *Acidilobus saccharovorans* (pink) (1,499 coding sequences) and *Aeropyrum pernix* (yellow) (1,700 coding sequences).

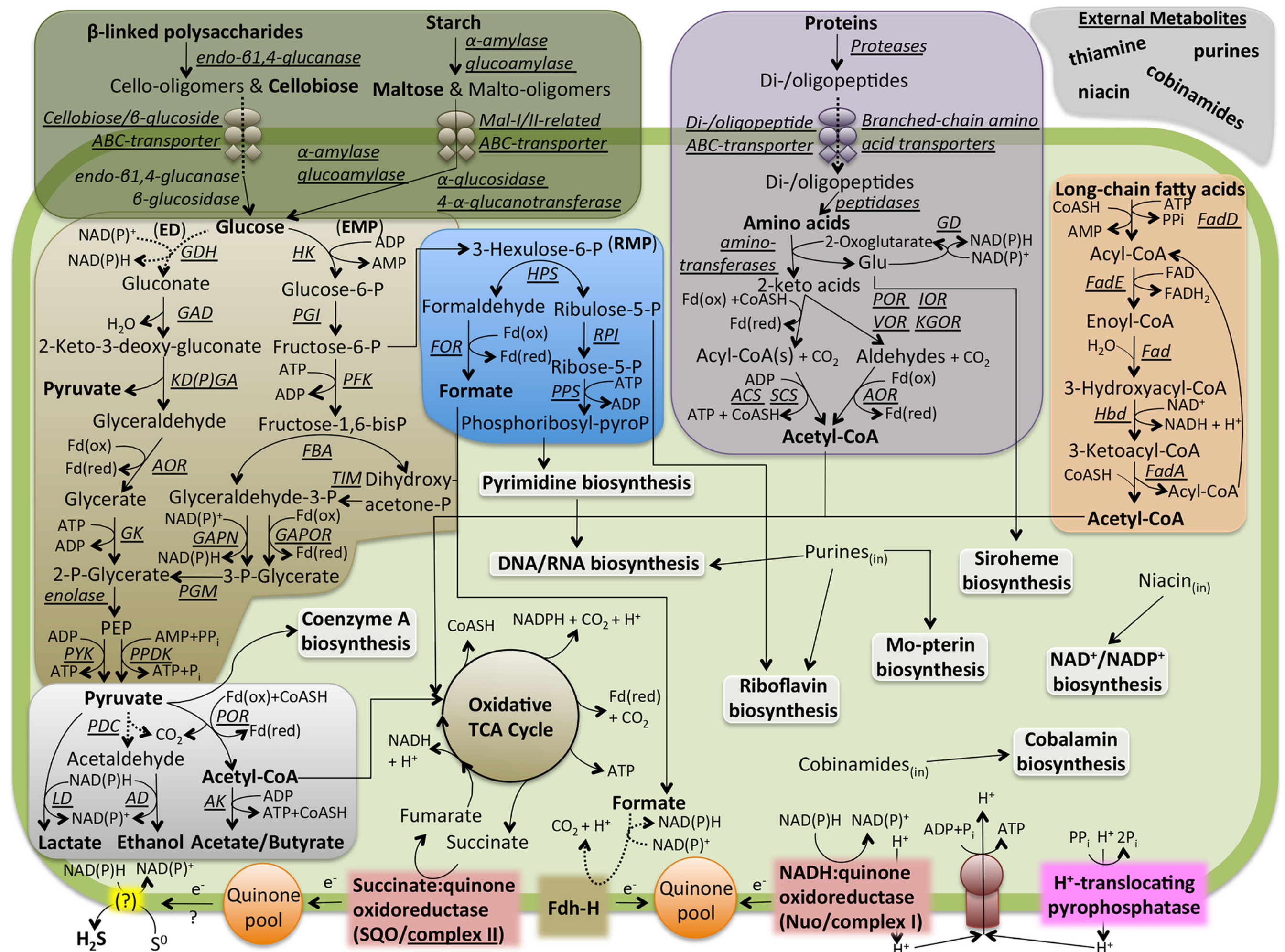

FIG 4 Metabolic potential of *Acidilobus*-like populations from YNP deduced from coding sequences obtained from metagenome assemblies. Dotted lines indicate enzymes not identified in all assemblies (see Table S4 in the supplemental material). GDH, glucose dehydrogenase; GAD, gluconate dehydratase; AOR, aldehyde:ferredoxin oxidoreductase; PYK, pyruvate kinase; PPDK, pyruvate, phosphate dikinase; HK, ADP-dependent hexokinase; PGI, phosphoglucose isomerase; PFK, ATP-dependent phosphofructokinase; TIM, triosephosphate isomerase; GK, glycerate kinase; RMP, ribulose monophosphate pathway; HPS, 3-hexulose-6-phosphate synthase; RP I, ribose-5-phosphate isomerase; PPS, phosphorybosyl pyrophosphate synthase; CoASH, coenzyme A-SH; PDC, pyruvate decarboxylase; LD, lactate dehydrogenase; AD, alcohol dehydrogenase; AK, acetate/butyrate kinase; GD, glutamate dehdyrogenase; ACS, acetyl-CoA synthetase; SCS, succinyl-CoA synthetase.

catabolism (degradation of starch and β-linked polysaccharides [58, 59]), and oxidation were also identified in all four *Acidilobus*-like assemblies (Fig. 4; see also Table S4 in the supplemental material). Coding sequences for proteins implicated in the fermentation of pyruvate to lactate, ethanol, and acetyl coenzyme A (acetyl-CoA) (or either butyrate or acetate), as well as formate dehydrogenases responsible for conversion of formate to CO_2, were identified (Fig. 4; see also Table S4 in the supplemental material). Multiple alcohol dehydrogenases were identified in all sites: 5 copies in MG and CIS, 4 copies in JCHS, and 3 copies in OSP.

The *Acidilobus*-like populations also contain genes coding for esterases and proteins used in the β-oxidation of long-chain fatty acids, which is currently identified in only three archaea, including *Archaeoglobus fulgidus* (60), *Vulcanisaeta moutnovskia* (61), and *Ac. saccharovorans* (11). The YNP *Acidilobus* populations also contain genes involved in archaeol biosynthesis, including isopentenyl-diphosphate delta-isomerase and geranylgeranyl pyrophosphate synthase (GGPP). The first aspartate-rich motif (FARM) in GGPP is identical to the GGPP of *Sulfolobus acidocaldarius* (62), which synthesizes a C_{20}-C_{20} ether lipid. This is in contrast to the rare C_{25}-C_{25} ether lipids identified in *Ae. pernix* (63, 64) and a novel archaeal population from candidate phylum *Geoarchaeota* (65), also identified as an abundant community member in OSP (29) (see Fig. S1 in the supplemental material). Complete Embden-Meyerhof (EM) and Entner-Doudoroff (ED) pathways were identified, and the ED pathway may be important for avoiding heat-labile intermediates (66). The EM pathway provides fructose-6-phosphate for the ribulose monophosphate pathway, consistent with genes identified in *Ac. saccharovorans* (11).

The fixation of carbon dioxide has been shown to occur in only two cultured isolates of the *Desulfurococcales* (*I. hospitalis* and *Pyrolobus fumarii*) via the dicarboxylase/4-hydroxybutyrate (DC/HB) pathway (19, 67, 68). However, only one of three key

"marker" genes for this pathway was identified in the *Acidilobus*-like populations from YNP. Two copies of the pyruvate:ferredoxin oxidoreductase (POR) were identified, which are highly related (83 to 84% amino acid identity) to the POR or VOR enzyme in *Ac. saccharovorans* (11, 69). These proteins are distantly related (<45%) to the proposed CO_2-assimilating enzyme pyruvate synthase in *I. hospitalis* (19). A potential 4-hydroxybutyryl-CoA dehydratase (4HCD) gene (also abbreviated 4-BUDH) was identified; however, phylogenetic analyses of the deduced proteins show that these fall between type I and type II 4HCDs (see Fig. S7 in the supplemental material). The function of type II 4HCDs is unknown, but they may represent an ancestral version of the type I 4HCDs (70). Homologs of the third and critical protein in the DC/HP pathway (phosphoenolpyruvate [PEP] carboxylase) were not identified in any of the four YNP assemblies, which suggests that these populations cannot assimilate CO_2 via this mechanism.

A 2-oxoglutarate:ferredoxin oxidoreductase (KGOR) and an indolepyruvate:ferredoxin oxidoreductase (IOR) were identified in the YNP assemblies (Fig. 4; see also Table S4 in the supplemental material). These oxygen-sensitive enzyme complexes, including POR and VOR, catalyze the reversible reactions of 2-oxoacids (pyruvate and 2-ketoglutarate), aromatic 2-keto acids (indolepyruvate), or branched-chain 2-keto acids (2-ketoisovalerate) with CoA and oxidized ferredoxin, producing acetyl-CoA, CO_2, and reduced ferredoxin (69, 71, 72). Although the directionality of these enzymes cannot be determined by amino acid sequence comparisons alone, the identification of the POR, VOR, KGOR, and IOR gene complexes suggests that the indigenous *Acidilobus*-like populations utilize these pathways for the catabolism of amino acids to organic acids and their subsequent oxidation to acetyl-CoA for further oxidation in the tricarboxylic acid (TCA) cycle or for conversion to lactate, formate, ethanol, or butyrate/acetate during fermentation (Fig. 4). Acetyl-CoA can also be oxidized via the TCA cycle, which is present in each assembly.

(ii) Electron transport. The *Acidilobus*-like assemblies from YNP lack heme Cu oxidases (subunit 1) and all other components of aerobic terminal oxidase complexes (73, 74). Each assembly contained *cydA* genes, which encode subunit A of the cytochrome *bd* ubiquinol oxidase (CydAB). However, these genes are not associated with a "typical" bacterial *cydAB*, and like numerous *Crenarchaeota*, the *Acidilobus* populations contained contiguous genes encoding a large copy (~560 amino acids [aa]) and a small copy (~400 aa) of CydA (defined here as CydA [large copy] and CydA′ [small copy]). Deduced CydA′ proteins are not homologous to CydB, which suggests that CydAA′ complexes do not perform the same function as CydAB in *Escherichia coli* (75), which has been shown to exhibit low K_m values for the reduction of oxygen. CydB contains a heme *d*, which is thought to interact with one of the heme domains of CydA (heme b_{595}) to form the binding site required for oxygen reduction (31, 75). The CydA present in the *Acidilobus* populations shares 7 (of 13) amino acids with *E. coli* CydA that are proposed to be important for O_2 reduction. These included H19 (the heme b_{595} axial ligand), Glu445 (required for charge compensation of the b_{595}/d O_2-reducing site upon its full reduction by 2 e^-), and Glu99, Glu107, and Ser140 (components of the proton channel) (see reference 31 and references therein) (see Fig. S6 and Table S8 in the supplemental material). However, *Acidilobus* CydA′ shares only the conserved H19 and M393 (heme b_{558} axial ligands) with *E. coli* CydA (see Table S8 in the supplemental material), and no conservation was detected between CydA′ and *E. coli* CydB. Consequently, the deduced CydAA′ proteins are not similar enough to the CydAB proteins in bacteria to suggest identical function. It has been suggested that these types of proteins may serve to reduce strong oxidants (e.g., O_2, O_2^-, H_2O_2, and OH·) (31).

Many cultivated *Desulfurococcales* (and all known *Acidilobus* spp.) are stimulated by elemental sulfur and produce sulfide during growth; consequently, it has been implied that elemental sulfur serves as a terminal electron acceptor during chemoorganotrophic growth. A well-established group of proteins responsible for sulfur and/or polysulfide reduction includes the dimethyl sulfoxide (DMSO)-molybdopterin sulfur/polysulfide reductases (*sreA* and *psrA*) (15, 66, 76–78). It has been assumed that these same proteins are responsible for sulfur reduction in the *Desulfurococcales* (11, 15). A recent annotation of *Ac. saccharovorans* reported the presence of a sulfur reductase (*sreA*) gene responsible for the reduction of elemental sulfur (ASAC_1397) (11). However, detailed phylogenetic analysis of the catalytic subunit of this DMSO-molybdopterin protein clearly shows homology to other formate dehydrogenases (Fdh) (Fig. 5A). No precedent exists to suggest that formate dehydrogenases are involved in the reduction of sulfur. Formate dehydrogenases (Fdh-H-like) were identified in assemblies from MG and JCHS (92% and 89% amino acid identities to FdhH in *Ac. saccharovorans* and *Ac. sulfurireducens*, respectively). Moreover, all *Acidilobus* Fdh-H-like sequences share 14 (of 15) invariant amino acid residues involved in the coordination of the molybdenum atom, the two molybdopterin guanine dinucleotide cofactors, formate, and the Fe_4S_4 cluster identified in the *E. coli* formate dehydrogenase (Fdh-H) crystal (Fig. 5A) (79). The cytoplasmic orientation of the *Acidilobus* Fdh-H proteins is also similar to those in *E. coli* in that no twin-arginine (TAT) signal is present for transport across the cytoplasmic membrane. These observations suggest that the oxidation of formate is occurring intracellularly. In *E. coli*, Fdh-H catalyzes the oxidation of formate to carbon dioxide coupled to the reduction of H^+ via an associated hydrogenase (i.e., formate-hydrogen lyase). No hydrogenases (Ni-Fe, Fe-Fe, or nonmetal cofactor) were identified in any of the YNP *Acidilobus* assemblies, which is consistent with previous studies showing that H_2 is not produced during growth of *Acidilobus* isolates (17, 54). Moreover, formate did not support the growth of either *Ac. saccharovorans* (17) or *Acidilobus aceticus* (80).

No obvious modes of sulfur reduction were found in the *Acidilobus* populations from YNP, based on pathways and proteins characterized to date. An 11-gene cluster was identified in all four assemblies, which exhibits obvious similarity to a gene cluster in *Ac. saccharovorans* annotated as an Mbx complex (11). However, the NADH dehydrogenase subunit D was incorrectly annotated as an MbxL and is actually more similar (based on amino acid identity) to NuoD (81) (see Fig. S3 in the supplemental material). The Nuo complex in *Acidilobus* spp. appears more similar to respiration complex I (82). There is no precedent that the Nuo complex is involved in the reduction of elemental sulfur, and the gene order is conserved across different *Desulfurococcales* independent of elemental sulfur reduction (see Fig. S4 in the supplemental material). Genes encoding "pyridine nucleotide-disulfide oxidoreductases" (NSRs) (78) were identified in all *Acidilobus* assemblies, but these genes are present in most sequenced archaea (see Fig. S5 in the supplemental material), and their function is currently unknown. Without the requisite Mbx complex, or a putative elemen-

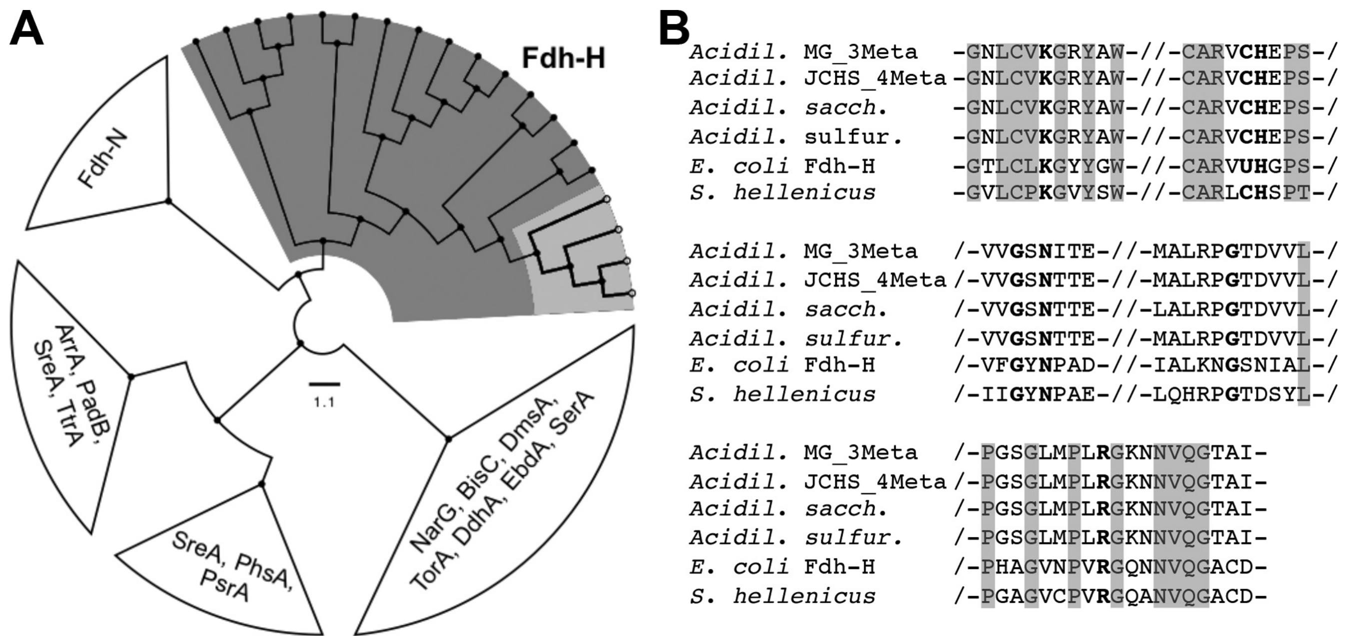

FIG 5 Phylogenetic analysis (A) and amino acid alignments (B) showing the relationship of DMSO-molybdopterin proteins identified in the *Acidilobus*-like assemblies to other formate dehydrogenase H (Fdh-H) proteins. (A) Phylogenetic tree of DMSO-molybdopterin proteins showing known formate dehydrogenases (Fdh-H) (dark gray) and *Acidilobus*-like Fdh-H entries from OSP, CIS, *Ac. saccharovorans*, and *Ac. sulfurireducens* (light gray). (B) Amino acid alignments showing conserved residues found to be invariant in Fdh-H proteins (bold) (79) (see Table S6 in the supplemental material) as well as additional conserved residues (shaded) (U, selenocysteine). Fdh-N, formate dehydrogenase N; BisC, biotin sulfoxide reductase; DmsA, dimethyl sulfoxide reductase; TorA, trimethylamine *N*-oxide reductase; NarG, membrane-bound nitrate reductase; DdhA, dimethylsulfide dehydrogenase; EbdA, ethylbenzene dehydrogenase; SerA, selenate reductase; PsrA/PhsA, polysulfide/polythionate reductase; SreA, elemental sulfur reductase; TtrA, tetrathionate reductase; ArrA, arsenate reductase. Individual entries and accession numbers are listed in Table S7 in the supplemental material.

tal sulfur response regulator, SurA (83, 84), the role of NSR proteins in sulfur reduction is unclear at this time. Genes similar to either the large subunits of sulfide dehydrogenase (*sudA*) or glutamate synthase (*gltD*) were also identified in the four *Acidilobus* populations (43% amino acid identity to both SudA and GltD characterized in *S. marinus* [16] and 35% amino acid identity to SudA of *Pyrococcus furiosus* [85]). Given that the YNP *Acidilobus* assemblies lacked a *sudB* gene necessary for sulfur reduction, this gene likely codes for glutamate synthase (GltD) rather than SudA.

(iii) Auxotrophy, vitamins, and cofactors. Genes involved in the synthesis of pyridoxine (vitamin B_6), biotin (vitamin B_7), or folic acid (vitamin B_9) were not identified in any of the YNP *Acidilobus* assemblies. This is consistent with the absence of proteins known to require these vitamins. Genomic sequence suggests that riboflavin (vitamin B_2) can be synthesized from ribulose-5-P (ribulose monophosphate pathway) and exogenous purines, while coenzyme A (vitamin B_5) can be synthesized directly from pyruvate. However, the *Acidilobus* populations from YNP are auxotrophic with respect to numerous other vitamins, metabolites, and cofactors essential for cellular processes (Fig. 4). No evidence for the biosynthesis of thiamine (vitamin B_1), niacin (vitamin B_3), and cobalamin (vitamin B_{12}) was observed for the four *Acidilobus* populations, although each assembly contained genes involved in processing, activating, and/or incorporating these vitamins (see Table S9 in the supplemental material). For example, genes coding for proteins involved in thiamine processing (thiamine ATP pyrophosphatase and thiamine monophosphate kinase) and transport (thiamine permease) were present and suggest that these populations require external sources of thiamine. Although several cobalamin biosynthesis genes were found (including four contiguous genes in a conserved gene cassette), the 30 genes required for *de novo* synthesis from glutamate were not identified (86). Instead, the *Acidilobus*-like populations may salvage corrinoid rings (cobinamides) and use these as precursors to synthesize an active form of cobalamin (87). Furthermore, the ATPase components of the ABC-type cobalamin transport system were identified, suggesting uptake of exogenous cobalamin (or intermediates) that can then be processed/activated for assimilation in the cytoplasm. The only evidence that cobalamin may be required by these populations is the presence of methylmalonyl CoA-mutase (*mcm*). Finally, although niacin (vitamin B_3) synthesis genes were not observed, this cofactor is required for NAD(P)H biosynthesis, and it is likely incorporated using nicotinic acid phosphoribosyl-transferase and NAD^+ synthetase (see Table S5 in the supplemental material).

Purine biosynthesis genes were not identified in any of the YNP *Acidilobus* assemblies, and this is consistent with other *Desulfurococcales* (e.g., *Ae. pernix*, *S. marinus*, and *H. butylicus*) that are unable to synthesize purines *de novo* (88). Purines are important precursors for the synthesis of the molybdopterin cofactor necessary for Fdh-H. Genes coding for Mo-pterin biosynthesis were identified in each *Acidilobus* assembly (see Table S4 in the supplemental material), which confirms the importance of Fdh-H (discussed above) despite the apparent need to import purines. Conversely, pyrimidine biosynthesis genes were present in each assembly. Cytosine, thymine, and uracil can be synthesized from UMP, which is initially produced by a set of reactions from phosphoribosyl-pyrophosphate and carbamoyl phosphate (see Table S4 in the supplemental material). Finally, genes for the *de novo* biosynthesis of aromatic amino acids, branch-chained

amino acids, lysine, arginine, and proline were not identified and are likely provided exogenously.

DISCUSSION

The metabolic similarity of four *Acidilobus* populations across sites exhibiting a pH range of 3.3 to 6.1 suggests that they perform a similar functional role across different types of microbial communities. The *Acidilobus* populations were abundant in sediments from sulfidic hot springs (MG, CIS, and JCHS) and low-sulfide, iron-oxide mats (OSP) (Fig. 1). Although a flux of oxygen is required to oxidize Fe(II) and form Fe(III)-oxide mats in OSP (7), these mats have been shown to be hypoxic below a depth of ~0.7 to 1 mm (89). Consequently, dissolved oxygen may be a more important variable (in addition to temperature) that defines the distribution of these *Acidilobus* populations, rather than high concentrations of elemental sulfur and/or dissolved sulfide. Although *Acidilobus* isolates are characterized as sulfur-respiring organisms, no definitive electron transport proteins associated with dissimilatory sulfur reduction have been identified in any of the genomes of these highly related organisms (11, 39). Consequently, their direct role in S cycling remains enigmatic.

A hallmark signature of all *Acidilobus* spp., including those from YNP, is the absence of respiratory heme Cu oxidases and the presence of *cydAA′* ubiquinol oxidase genes. These observations indicate that the *Acidilobus* populations do not respire oxygen using the common aerobic pathways and lack *cydB*, which is necessary for oxygen reduction in bacterial cytochrome *bd* ubiquinol oxidases (31, 75). Transcription of both *cydA* and *cydA′* was confirmed in several sites and is consistent with the high abundance of these populations in sulfidic sediments as well as suboxic Fe(III)-oxide mats (see Fig. S1 in the supplemental material). However, the structure, function, and role of CydAA′ proteins in the *Archaea* have not been determined, and no crystal structures of these proteins are available. It is unlikely that these genes are transcribed for no cellular purpose, and their presence in numerous members of the *Crenarchaeota* appears to be especially important in organisms that flourish in sulfidic and/or hypoxic habitats. The cotranscription of the *cydA* and *cydA′* genes as well as *fdhF* genes in environmental samples is consistent with the hypoxic conditions in which these populations are found and further supports a common functional role of these organisms in microbial community function. The fermentation of complex organic substrates to formate (CO_2), ethanol, lactate, acetate, and/or butyrate reveals electron transfer mechanisms that do not require direct reduction of elemental sulfur. The fermentation of yeast extract has been observed in controlled studies in the absence of elemental sulfur, using either *Ac. saccharovorans* (17) or *Ac. aceticus* (80). Importantly, this may explain a common functional role of this phylotype across both elemental sulfur and Fe(III)-oxide sediment communities in YNP (7).

The conservation of genomic traits among four *de novo Acidilobus*-like assemblies from YNP suggests that a “core” genome for this population is ~1,400 genes (Table 1 and Fig. 3B), which are also found in *Ac. saccharovorans*. *Acidilobus*-like organisms are distributed globally (e.g., YNP, Kamchatka, Japan, New Zealand, and Iceland) and are usually found in microbial communities containing other members of the phylum *Crenarchaeota*, including organisms within the orders *Thermoproteales* and *Sulfolobales* (22–28) (see Fig. S1 in the supplemental material), several of which have been shown to fix carbon dioxide and may provide *Acidilobus* organisms with fermentable substrates. The exact sources of complex carbon utilized by *Acidilobus*-like populations cannot be ascertained from the sequence data reported here but likely include a mixture of carbon fixed by other members of these communities (e.g., other *Crenarchaeota* that use the 3HP/4HB or DC/HD carbon dioxide fixation pathways [29]) as well as organic carbon from geothermal or landscape sources. The *Acidilobus*-like populations evaluated here range in abundance in S sediments and Fe(III)-oxide microbial mats from as low as a few percent to as high as 70% in Monarch Geyser sediments. Factors that may contribute to this variation, in addition to site conditions, include sample content (e.g., exact location and depth) and DNA extraction method, both of which can result in variations in observed population abundances. Genomic evidence suggests that the *Acidilobus*-like populations present in high-temperature, mildly acidic, hypoxic geothermal systems of YNP are dependent on other community members or environmental inputs for specific amino acids, purines, and several important vitamins and cofactors. Moreover, metabolic reconstruction suggests that these populations degrade and ferment complex sources of organic carbon and that they serve a common functional role in carbon cycling reactions important across different high-temperature microbial communities.

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REFERENCES

1. **Langner HW, Jackson CR, McDermott TR, Inskeep WP.** 2001. Rapid oxidation of arsenite in a hot spring ecosystem, Yellowstone National Park. Environ. Sci. Technol. **35:**3302–3309. http://dx.doi.org/10.1021/es0105562.
2. **Ball JW, McCleskey RB, Nordstrom DK, Holloway JM, Verplanck PL.** 2002. Water-chemistry data for selected springs, geysers, and streams in Yellowstone National Park, Wyoming 1999-2000. US Geological Survey open-file report 02-382. US Geological Survey, Boulder, CO.
3. **Amend JP, Shock EL.** 2001. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. FEMS Microbiol. Rev. **25:**175–243. http://dx.doi.org/10.1111/j.1574-6976.2001.tb00576.x.
4. **Inskeep WP, Ackerman GG, Taylor WP, Kozubal MA, Korf SL, Macur RE.** 2005. On the energetics of chemolithotrophy in nonequilibrium systems: case studies of geothermal springs in Yellowstone National Park. Geobiology **3:**297–317. http://dx.doi.org/10.1111/j.1472-4669.2006.00059.x.
5. **Newman DK, Banfield JF.** 2002. Geomicrobiology: how molecular-scale interactions underpin biogeochemical systems. Science **296:**1071–1077. http://dx.doi.org/10.1126/science.1010716.
6. **Inskeep WP, Macur RE, Harrison G, Bostick BC, Fendorf S.** 2004. Biomineralization of As(V)-hydrous ferric oxyhydroxide in microbial mats of an acid-sulfate-chloride geothermal spring, Yellowstone National Park. Geochim. Cosmochim. Acta **68:**3141–3155. http://dx.doi.org/10.1016/j.gca.2003.09.020.
7. **Kozubal MA, Macur RE, Jay ZJ, Beam JP, Malfatti SA, Tringe SG, Kocar BD, Borch T, Inskeep WP.** 2012. Microbial iron cycling in acidic geothermal springs of Yellowstone National Park: integrating molecular surveys, geochemical processes, and isolation of novel Fe-active microor-

ganisms. Front. Microbiol. **3**:109. http://dx.doi.org/10.3389/fmicb.2012.00109.
8. **Huber H, Stetter KO.** 2006. Desulfurococcales, p 52–68. *In* Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), The prokaryotes. Springer, New York, NY.
9. **Huber H, Huber R, Stetter KO.** 2006. Thermoproteales, p 10–22. *In* Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), The prokaryotes. Springer, New York, NY.
10. **Huber H, Prangishvili D.** 2006. Sulfolobales, p 23–51. *In* Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), The prokaryotes. Springer, New York, NY.
11. **Mardanov AV, Svetlitchnyi VA, Beletsky AV, Prokofeva MI, Bonch-Osmolovskaya EA, Ravin NV, Skryabin KG.** 2010. The genome sequence of the crenarchaeon *Acidilobus saccharovorans* supports a new order, *Acidilobales*, and suggests an important ecological role in terrestrial acidic hot springs. Appl. Environ. Microbiol. **76**:5652–5657. http://dx.doi.org/10.1128/AEM.00599-10.
12. **Kawarabayasi Y, Hino Y, Horikawa H, Yamazaki S, Haikawa Y, Jin-no K, Takahashi M, Sekine M, Baba S, Ankai A, Kosugi H, Hosoyama A, Fukui S, Nagai Y, Nishijima K, Nakazawa H, Takamiya M, Masuda S, Funahashi T, Tanaka T, Kudoh Y, Yamazaki J, Kushida N, Oguchi A, Kikuchi H.** 1999. Complete genome sequence of an aerobic hyperthermophilic crenarchaeon, *Aeropyrum pernix* K1. DNA Res. **6**:83–101, 145–152.
13. **Ravin NV, Mardanov AV, Beletsky AV, Kublanov IV, Kolganova TV, Lebedinsky AV, Chernyh NA, Bonch-Osmolovskaya EA, Skryabin KG.** 2009. Complete genome sequence of the anaerobic, protein-degrading hyperthermophilic crenarchaeon *Desulfurococcus kamchatkensis*. J. Bacteriol. **191**:2371–2379. http://dx.doi.org/10.1128/JB.01525-08.
14. **Brügger K, Chen L, Stark M, Zibat A, Redder P, Ruepp A, Awayez M, She Q, Garrett RA, Klenk H-P.** 2007. The genome of *Hyperthermus butylicus*: a sulfur-reducing, peptide fermenting, neutrophilic crenarchaeote growing up to 108 degrees C. Archaea **2**:127–135. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2686385/.
15. **Podar M, Anderson IJ, Makarova KS, Elkins JG, Ivanova N, Wall MA, Lykidis A, Mavromatis K, Sun H, Hudson ME, Chen W, Deciu C, Hutchison D, Eads JR, Anderson A, Fernandes F, Szeto E, Lapidus A, Kyrpides NC, Saier MH, Richardson PM, Rachel R, Huber H, Eisen JA, Koonin EV, Keller M, Stetter KO.** 2008. A genomic analysis of the archaeal system *Ignicoccus hospitalis-Nanoarchaeum equitans*. Genome Biol. **9**:R158. http://dx.doi.org/10.1186/gb-2008-9-11-r158.
16. **Anderson IJ, Dharmarajan L, Rodriguez J, Hooper S, Porat I, Ulrich LE, Elkins JG, Mavromatis K, Sun H, Land M, Lapidus A, Lucas S, Barry K, Huber H, Zhulin IB, Whitman WB, Mukhopadhyay B, Woese C, Bristow J, Kyrpides N.** 2009. The complete genome sequence of *Staphylothermus marinus* reveals differences in sulfur metabolism among heterotrophic Crenarchaeota. BMC Genomics **10**:145–158. http://dx.doi.org/10.1186/1471-2164-10-145.
17. **Prokofeva MI, Kostrikina NA, Kolganova TV, Tourova TP, Lysenko AM, Lebedinsky AV, Bonch-Osmolovskaya EA.** 2009. Isolation of the anaerobic thermoacidophilic crenarchaeote *Acidilobus saccharovorans* sp. nov. and proposal of *Acidilobales* ord. nov., including *Acidilobaceae* fam. nov. and *Caldisphaeraceae* fam. nov. Int. J. Syst. Evol. Microbiol. **59**:3116–3122. http://dx.doi.org/10.1099/ijs.0.010355-0.
18. **Brochier-Armanet C, Forterre P, Gribaldo S.** 2011. Phylogeny and evolution of the Archaea: one hundred genomes later. Curr. Opin. Microbiol. **14**:274–281. http://dx.doi.org/10.1016/j.mib.2011.04.015.
19. **Huber H, Gallenberger M, Jahn U, Eylert E, Berg IA, Kockelkorn D, Eisenreich W, Fuchs G.** 2008. A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic archaeum *Ignicoccus hospitalis*. Proc. Natl. Acad. Sci. U. S. A. **105**:7851–7856. http://dx.doi.org/10.1073/pnas.0801043105.
20. **Meyer-Dombard DR, Shock EL, Amend JP.** 2005. Archaeal and bacterial communities in geochemically diverse hot springs of Yellowstone National Park, USA. Geobiology **3**:211–227. http://dx.doi.org/10.1111/j.1472-4669.2005.00052.x.
21. **Macur RE, Jay ZJ, Taylor WP, Kozubal MA, Kocar BD, Inskeep WP.** 2013. Microbial community structure and sulfur biogeochemistry in mildly-acidic sulfidic geothermal springs in Yellowstone National Park. Geobiology **11**:86–99. http://dx.doi.org/10.1111/gbi.12015.
22. **Takai K, Horikoshi K.** 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. Genetics **152**:1285–1297.
23. **Hoek J, Banta A, Hubler F, Reysenbach A.** 2003. Microbial diversity of a sulphide spire located in the Edmond deep-sea hydrothermal vent field on the Central Indian Ridge. Geobiology **1**:119–127. http://dx.doi.org/10.1046/j.1472-4669.2003.00015.x.
24. **Nercessian O, Reysenbach A-L, Prieur D, Jeanthon C.** 2003. Archaeal diversity associated with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13 degrees N). Environ. Microbiol. **5**:492–502. http://dx.doi.org/10.1046/j.1462-2920.2003.00437.x.
25. **Perevalova AA, Kolganova TV, Birkeland N-K, Schleper C, Bonch-Osmolovskaya EA, Lebedinsky AV.** 2008. Distribution of Crenarchaeota representatives in terrestrial hot springs of Russia and Iceland. Appl. Environ. Microbiol. **74**:7620–7628. http://dx.doi.org/10.1128/AEM.00972-08.
26. **Kublanov IV, Perevalova AA, Slobodkina GB, Lebedinsky AV, Bidzhieva SK, Kolganova TV, Kaliberda EN, Rumsh LD, Haertlé T, Bonch-Osmolovskaya EA.** 2009. Biodiversity of thermophilic prokaryotes with hydrolytic activities in hot springs of Uzon Caldera, Kamchatka (Russia). Appl. Environ. Microbiol. **75**:286–291. http://dx.doi.org/10.1128/AEM.00607-08.
27. **Nunoura T, Oida H, Nakaseama M, Kosaka A, Ohkubo SB, Kikuchi T, Kazama H, Hosoi-Tanabe S, Nakamura K, Kinoshita M, Hirayama H, Inagaki F, Tsunogai U, Ishibashi J, Takai K.** 2010. Archaeal diversity and distribution along thermal and geochemical gradients in hydrothermal sediments at the Yonaguni Knoll IV hydrothermal field in the Southern Okinawa Trough. Appl. Environ. Microbiol. **76**:1198–1211. http://dx.doi.org/10.1128/AEM.00924-09.
28. **Mardanov AV, Gumerov VM, Beletsky AV, Perevalova AA, Karpov GA, Bonch-Osmolovskaya EA, Ravin NV.** 2011. Uncultured archaea dominate in the thermal groundwater of Uzon Caldera, Kamchatka. Extremophiles **15**:365–372. http://dx.doi.org/10.1007/s00792-011-0368-1.
29. **Inskeep WP, Jay ZJ, Herrgard MJ, Kozubal MA, Rusch DB, Tringe SG, Boyd ES, Spear JR, Roberto FF.** 2013. Phylogenetic and functional analysis of metagenome sequence from high-temperature archaeal habitats demonstrate linkages between metabolic potential and geochemistry. Front. Microbiol. **4**:95. http://dx.doi.org/10.3389/fmicb.2013.00095.
30. **Sako Y, Nomura N, Uchida A, Ishida Y, Morii H, Koga Y, Hoaki T, Maruyama T.** 1996. *Aeropyrum pernix* gen. nov., sp. nov., a novel aerobic hyperthermophilic archaeon growing at temperatures up to 100 degrees C. Int. J. Syst. Bacteriol. **46**:1070–1077. http://dx.doi.org/10.1099/00207713-46-4-1070.
31. **Borisov VB, Gennis RB, Hemp J, Verkhovsky MI.** 2011. The cytochrome *bd* respiratory oxygen reductases. Biochim. Biophys. Acta **1807**:1398–1413. http://dx.doi.org/10.1016/j.bbabio.2011.06.016.
32. **Nisbet EG, Sleep NH.** 2001. The habitat and nature of early life. Nature **409**:1083–1091. http://dx.doi.org/10.1038/35059210.
33. **Stetter KO.** 2006. Hyperthermophiles in the history of life. Philos. Trans. R. Soc. B Biol. Sci. **361**:1837–1843. http://dx.doi.org/10.1098/rstb.2006.1907.
34. **Blank CE.** 2009. Not so old Archaea—the antiquity of biogeochemical processes in the archaeal domain of life. Geobiology **7**:495–514. http://dx.doi.org/10.1111/j.1472-4669.2009.00219.x.
35. **Inskeep WP, Jay ZJ, Tringe SG, Herrgard MJ, Rusch DB.** 2013. The YNP metagenome project: environmental parameters responsible for microbial distribution in the Yellowstone geothermal ecosystem. Front. Microbiol. **4**:67. http://dx.doi.org/10.3389/fmicb.2013.00067.
36. **McCleskey RB, Ball JW, Nordstrom DK, Holloway JM, Taylor HE.** 2004. Water-chemistry data for selected hot springs, geysers, and streams in Yellowstone National Park, Wyoming, 2001-2002. US Geological Survey open-file report 2004-1316. US Geological Survey, Boulder, CO.
37. **APHA.** 1998. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.
38. **Sambrook J, Russell DW.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
39. **Inskeep WP, Rusch DB, Jay ZJ, Herrgard MJ, Kozubal MA, Richardson TH, Macur RE, Hamamura N, Jennings RM, Birren BW, Reysenbach A-L, Roberto F, Young M, Schwartz A, Boyd ES, Badger JH, Mathur EJ, Ortmann AC, Bateson M, Geesey G, Frazier M.** 2010. Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. PLoS One **5**:e9773. http://dx.doi.org/10.1371/journal.pone.0009773.
40. **Teeling H, Waldmann J, Lombardot T, Bauer M, Glöckner FO.** 2004. TETRA: a Web-service and a stand-alone program for the analysis and comparison of tetranucleotide usage patterns in DNA sequences. BMC Bioinformatics **5**:163–170. http://dx.doi.org/10.1186/1471-2105-5-163.
41. **Markowitz VM, Mavromatis K, Ivanova NN, Chen I-MA, Chu K,**

Kyrpides NC. 2009. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics 25:2271–2278. http://dx.doi.org/10.1093/bioinformatics/btp393.

42. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.
43. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797. http://dx.doi.org/10.1093/nar/gkh340.
44. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731–2739. http://dx.doi.org/10.1093/molbev/msr121.
45. Matte-Tailliez O, Brochier C, Forterre P, Philippe H. 2002. Archaeal phylogeny based on ribosomal proteins. Mol. Biol. Evol. 19:631–639. http://dx.doi.org/10.1093/oxfordjournals.molbev.a004122.
46. Slesarev AI, Mezhevaya KV, Makarova KS, Polushin NN, Shcherbinina OV, Shakhova VV, Belova GI, Aravind L, Natale DA, Rogozin IB, Tatusov RL, Wolf YI, Stetter KO, Malykh AG, Koonin EV, Kozyavkin SA. 2002. The complete genome of hyperthermophile *Methanopyrus kandleri* AV19 and monophyly of archaeal methanogens. Proc. Natl. Acad. Sci. U. S. A. 99:4644–4649. http://dx.doi.org/10.1073/pnas.032671499.
47. Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, Barnstead M, Beeson KY, Bibbs L, Bolanos R, Keller M, Kretz K, Lin X, Mathur E, Ni J, Podar M, Richardson T, Sutton GG, Simon M, Söll D, Stetter KO, Short JM, Noordewier M. 2003. The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. Proc. Natl. Acad. Sci. U. S. A. 100:12984–12988. http://dx.doi.org/10.1073/pnas.1735403100.
48. Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P. 2008. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nat. Rev. Microbiol. 6:245–252. http://dx.doi.org/10.1038/nrmicro1852.
49. Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S, Wu D, Eisen JA, Hoffman JM, Remington K, Beeson K, Tran B, Smith H, Baden-Tillson H, Stewart C, Thorpe J, Freeman J, Andrews-Pfannkoch C, Venter JE, Li K, Kravitz S, Heidelberg JF, Utterback T, Rogers Y-H, Falcón LI, Souza V, Bonilla-Rosso G, Eguiarte LE, Karl DM, Sathyendranath S, Platt T, Bermingham E, Gallardo V, Tamayo-Castillo G, Ferrari MR, Strausberg RL, Nealson K, Friedman R, Frazier M, Venter JC. 2007. The Sorcerer II global ocean sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS Biol. 5:e77. http://dx.doi.org/10.1371/journal.pbio.0050077.
50. Darling AE, Tritt A, Eisen JA, Facciotti MT. 2011. Mauve assembly metrics. Bioinformatics 27:2756–2757. http://dx.doi.org/10.1093/bioinformatics/btr451.
51. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. http://dx.doi.org/10.1371/journal.pone.0011147.
52. Carver TJ, Rutherford KM, Berriman M, Rajandream M-A, Barrell BG, Parkhill J. 2005. ACT: the Artemis comparison tool. Bioinformatics 21:3422–3423. http://dx.doi.org/10.1093/bioinformatics/bti553.
53. Kato S, Itoh T, Yamagishi A. 2011. Archaeal diversity in a terrestrial acidic spring field revealed by a novel PCR primer targeting archaeal 16S rRNA genes. FEMS Microbiol. Lett. 319:34–43. http://dx.doi.org/10.1111/j.1574-6968.2011.02267.x.
54. Boyd ES, Jackson RA, Encarnacion G, Zahn JA, Beard T, Leavitt WD, Pi Y, Zhang CL, Pearson A, Geesey GG. 2007. Isolation, characterization, and ecology of sulfur-respiring Crenarchaea inhabiting acid-sulfate-chloride-containing geothermal springs in Yellowstone National Park. Appl. Environ. Microbiol. 73:6669–6677. http://dx.doi.org/10.1128/AEM.01321-07.
55. Macur RE, Langner HW, Kocar BD, Inskeep WP. 2004. Linking geochemical processes with microbial community analysis: successional dynamics in an arsenic-rich, acid-sulphate-chloride geothermal spring. Geobiology 2:163–177. http://dx.doi.org/10.1111/j.1472-4677.2004.00032.x.
56. Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a Web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res. 35:W52–W57. http://dx.doi.org/10.1093/nar/gkm360.
57. Chaisson M, Pevzner P, Tang H. 2004. Fragment assembly with short reads. Bioinformatics 20:2067–2074. http://dx.doi.org/10.1093/bioinformatics/bth205.
58. de Vos WM, Kengen SWM, Voorhorst WGB, van der Oost J. 1998. Sugar utilization and its control in hyperthermophiles. Extremophiles 2:201–205. http://dx.doi.org/10.1007/s007920050061.
59. Koning SM, Albers S-V, Konings WN, Driessen AJM. 2002. Sugar transport in (hyper)thermophilic archaea. Res. Microbiol. 153:61–67. http://dx.doi.org/10.1016/S0923-2508(01)01289-X.
60. Klenk H-P, Clayton RA, Tomb J-F, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, Richardson DL, Kerlavage AR, Graham DE, Kyrpides NC, Fleischmann RD, Quackenbush J, Lee NH, Sutton GG, Gill S, Kirkness EF, Dougherty BA, McKenney K, Adams MD, Loftus B, Peterson S, Reich CI, McNeil LK, Badger JH, Glodek A, Zhou L, Overbeek R, Gocayne JD, Weidman JF, McDonald L, Utterback T, Cotton MD, Spriggs T, Artiach P, Kaine BP, Sykes SM, Sadow PW, D'Andrea KP, Bowman C, Fujii C, Garland SA, Mason TM, Olsen GJ, Fraser CM, Smith HO, Woese CR, Venter JC. 1997. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature 390:364–370. http://dx.doi.org/10.1038/37052.
61. Gumerov VM, Mardanov AV, Beletsky AV, Prokofeva MI, Bonch-Osmolovskaya EA, Ravin NV, Skryabin KG. 2011. Complete genome sequence of "*Vulcanisaeta moutnovskia*" strain 768-28, a novel member of the hyperthermophilic crenarchaeal genus *Vulcanisaeta*. J. Bacteriol. 193:2355–2356. http://dx.doi.org/10.1128/JB.00237-11.
62. Hemmi H, Ikejiri S, Yamashita S, Nishino T. 2002. Novel medium-chain prenyl diphosphate synthase from the thermoacidophilic archaeon *Sulfolobus solfataricus*. J. Bacteriol. 184:615–620. http://dx.doi.org/10.1128/JB.184.3.615-620.2002.
63. Morii H, Yagi H, Akutsu H, Nomura N, Sako Y, Koga Y. 1999. A novel phosphoglycolipid archaetidyl(glucosyl)inositol with two sesterterpanyl chains from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* K1. Biochim. Biophys. Acta 1436:426–436.
64. Matsumi R, Atomi H, Driessen AJM, van der Oost J. 2011. Isoprenoid biosynthesis in Archaea—biochemical and evolutionary implications. Res. Microbiol. 162:39–52. http://dx.doi.org/10.1016/j.resmic.2010.10.003.
65. Kozubal MA, Romine M, Jennings RM, Jay ZJ, Tringe SG, Rusch DB, Beam JP, McCue LA, Inskeep WP. 2013. Geoarchaeota: a new candidate phylum in the Archaea from high-temperature acidic iron mats in Yellowstone National Park. ISME J. 7:622–634.
66. Siebers B, Tjaden B, Michalke K, Dorr C, Ahmed H, Zaparty M, Gordon P, Sensen CW, Zibat A, Klenk H-P, Schuster SC, Hensel R. 2004. Reconstruction of the central carbohydrate metabolism of *Thermoproteus tenax* by use of genomic and biochemical data. J. Bacteriol. 186:2179–2194. http://dx.doi.org/10.1128/JB.186.7.2179-2194.2004.
67. Ramos-Vera WH, Berg IA, Fuchs G. 2009. Autotrophic carbon dioxide assimilation in Thermoproteales revisited. J. Bacteriol. 191:4286–4297. http://dx.doi.org/10.1128/JB.00145-09.
68. Berg IA, Ramos-Vera WH, Petri A, Huber H, Fuchs G. 2010. Study of the distribution of autotrophic CO_2 fixation cycles in Crenarchaeota. Microbiology 156:256–269. http://dx.doi.org/10.1099/mic.0.034298-0.
69. Kletzin A, Adams MWW. 1996. Molecular and phylogenetic characterization of pyruvate and 2-ketoisovalerate ferredoxin oxidoreductases from *Pyrococcus furiosus* and pyruvate ferredoxin oxidoreductase from *Thermotoga maritima*. J. Bacteriol. 178:248–257.
70. Berg IA, Kockelkorn D, Buckel W, Fuchs G. 2007. A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. Science 318:1782–1786. http://dx.doi.org/10.1126/science.1149976.
71. Mai X, Adams MWW. 1994. Indolepyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon *Pyrococcus furiosus*. A new enzyme involved in peptide fermentation. J. Biol. Chem. 269:16726–16732.
72. Mai X, Adams MWW. 1996. Characterization of a fourth type of 2-keto acid-oxidizing enzyme from a hyperthermophilic archaeon: 2-ketoglutarate ferredoxin oxidoreductase from *Thermococcus litoralis*. J. Bacteriol. 178:5890–5896.
73. García-Horsman JA, Barquera B, Rumbley J, Ma J, Gennis RB. 1994. The superfamily of heme-copper respiratory oxidases. J. Bacteriol. 176:5587–5600.
74. Sousa FL, Alves RJ, Pereira-Leal JB, Teixeira M, Pereira MM. 2011. A bioinformatics classifier and database for heme-copper oxygen reductases. PLoS One 6:e19117. http://dx.doi.org/10.1371/journal.pone.0019117.

75. **Jünemann S.** 1997. Cytochrome *bd* terminal oxidase. Biochim. Biophys. Acta **1321:**107–127. http://dx.doi.org/10.1016/S0005-2728(97)00046-7.
76. **McEwan AG, Ridge JP, McDevitt CA, Hugenholtz P.** 2002. The DMSO-reductase family of microbial molybdenum enzymes; molecular properties and role in the dissimilatory reduction of toxic elements. Geomicrobiol. J. **19:**3–21. http://dx.doi.org/10.1080/014904502317246138.
77. **Laska S, Lottspeich F, Kletzin A.** 2003. Membrane-bound hydrogenase and sulfur reductase of the hyperthermophilic and acidophilic archaeon *Acidianus ambivalens*. Microbiology **149:**2357–2371. http://dx.doi.org/10.1099/mic.0.26455-0.
78. **Schut GJ, Bridger SL, Adams MWW.** 2007. Insights into the metabolism of elemental sulfur by the hyperthermophilic archaeon *Pyrococcus furiosus*: characterization of a coenzyme A-dependent NAD(P)H sulfur oxidoreductase. J. Bacteriol. **189:**4431–4441. http://dx.doi.org/10.1128/JB.00031-07.
79. **Boyington JC, Gladyshev VN, Khangulov SV, Stadtman TC, Sun PD.** 1997. Crystal structure of formate dehydrogenase H: catalysis involving Mo, molybdopterin, selenocysteine, and an Fe_4S_4 cluster. Science **275:** 1305–1308. http://dx.doi.org/10.1126/science.275.5304.1305.
80. **Prokofeva MI, Miroshnichenko ML, Kostrikina NA, Chernyh NA, Kuznetsov BB, Tourova TP, Bonch-Osmolovskaya EA.** 2000. *Acidilobus aceticus* gen. nov., sp. nov., a novel anaerobic thermoacidophilic archaeon from continental hot vents in Kamchatka. Int. J. Syst. Evol. Microbiol. **50:**2001–2008. http://dx.doi.org/10.1099/0020771350-6-2001.
81. **Schut GJ, Boyd ES, Peters JW, Adams MWW.** 2013. The modular respiratory complexes involved in hydrogen and sulfur metabolism by heterotrophic hyperthermophilic archaea and their evolutionary implications. FEMS Microbiol. Rev. **37:**182–203. http://dx.doi.org/10.1111/j.1574-6976.2012.00346.x.
82. **Friedrich T, Scheide D.** 2000. The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases. FEBS Lett. **479:**1–5. http://dx.doi.org/10.1016/S0014-5793(00)01867-6.
83. **Lipscomb GL, Keese AM, Cowart DM, Schut GJ, Thomm M, Adams MWW, Scott RA.** 2009. SurR: a transcriptional activator and repressor controlling hydrogen and elemental sulphur metabolism in *Pyrococcus furiosus*. Mol. Microbiol. **71:**332–349. http://dx.doi.org/10.1111/j.1365-2958.2008.06525.x.
84. **Bridger SL, Clarkson SM, Stirrett K, DeBarry MB, Lipscomb GL, Schut GJ, Westpheling J, Scott RA, Adams MWW.** 2011. Deletion strains reveal metabolic roles for key elemental sulfur-responsive proteins in *Pyrococcus furiosus*. J. Bacteriol. **193:**6498–6504. http://dx.doi.org/10.1128/JB.05445-11.
85. **Ma K, Adams MWW.** 1994. Sulfide dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus*: a new multifunctional enzyme involved in the reduction of elemental sulfur. J. Bacteriol. **176:**6509–6517.
86. **Martens J-H, Barg H, Warren M, Jah D.** 2002. Microbial production of vitamin B 12. Appl. Microbiol. Biotechnol. **58:**275–285. http://dx.doi.org/10.1007/s00253-001-0902-7.
87. **Escalante-Semerena JC.** 2007. Conversion of cobinamide into adenosylcobamide in bacteria and archaea. J. Bacteriol. **189:**4555–4560. http://dx.doi.org/10.1128/JB.00503-07.
88. **Brown AM, Hoopes SL, White RH, Sarisky CA.** 2011. Purine biosynthesis in archaea: variations on a theme. Biol. Direct **6:**63. http://dx.doi.org/10.1186/1745-6150-6-63.
89. **Bernstein HC, Beam JP, Kozubal MA, Carlson RP, Inskeep WP.** 2013. *In situ* analysis of oxygen consumption and diffusive transport in high-temperature acidic iron-oxide microbial mats. Environ. Microbiol. **15:** 2360–2370. http://dx.doi.org/10.1111/1462-2920.12109.
90. **Zillig W, Holz I, Janekovic D, Klenk H-P, Imsel E, Trent J, Wunderl S, Forjaz VH, Coutinho R, Ferreira T.** 1990. *Hyperthermus butylicus*, a hyperthermophilic sulfur-reducing archaebacterium that ferments peptides. J. Bacteriol. **172:**3959–3965.
91. **Huber H, Burggraf S, Mayer T, Wyschkony I, Rachel R, Stetter KO.** 2000. *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic *Archaea*, represented by two new species, *Ignicoccus islandicus* sp. nov. and *Ignicoccus pacificus* sp. nov. Int. J. Syst. Evol. Microbiol. **50:**2093–2100. http://dx.doi.org/10.1099/00207713-50-6-2093.
92. **Paper W, Jahn U, Hohn MJ, Kronner M, Näther DJ, Burghardt T, Rachel R, Stetter KO, Huber H.** 2007. *Ignicoccus hospitalis* sp. nov., the host of '*Nanoarchaeum equitans*.' Int. J. Syst. Evol. Microbiol. **57:**803–808. http://dx.doi.org/10.1099/ijs.0.64721-0.