

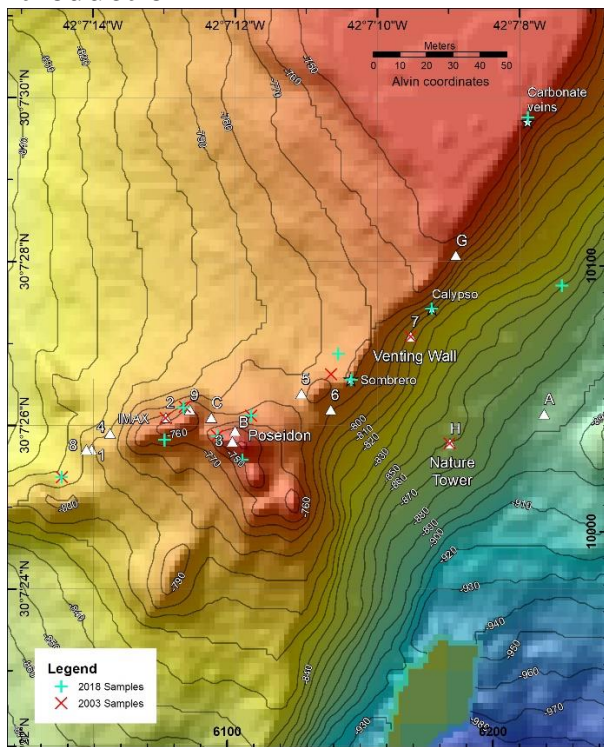


## LOST CITY THERMODESULFOVIBRIONALES, UBA665 SPP.

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Thermodesulfovibrionales are abundant at the Lost City Hydrothermal Field (LCHF) and may be an integral member of this hydrothermal ecosystem. Previous expeditions to the Lost City (LC) predicted that sulfur-reducing microorganisms are the foundation of the subsurface microbial ecosystem underlying the LCHF, but previous studies had not yet identified such organisms. Through metagenomic methods we were able to investigate candidate organisms, including extremophilic sulfur reducers such as the LC Thermodesulfovibrionales. Results suggest a range of potential electron donors including  $H_2$ , formate, and lactate. These electron donors were identified based on genes encoding for multiple hydrogenases, formate dehydrogenases, a lactate dehydrogenase, and other associated proteins. Encoded pathways suggest that this organism has autotrophic and heterotrophic modes, extending its metabolic flexibility. The bins representing this organism are important for enhancing our understanding of metabolic strategies found in the LCHF's ecosystem, in addition to other alkaline hydrothermal environments.

### Introduction



**Figure 1** Topographical map of the Lost City hydrothermal field (LCHF). Markers of interest include Calypso, Poseidon, IMAX, and Sombrero, abbreviated as CALY, PoNS and PoCH (Poseidon North Spire and Camel Humps), SOM1 and SOM2 (two sets of samples from the same site), and IMAX, respectively.

In 2018, the RV Atlantis cruise AT42-01 launched submersible *Jason* to explore the LCHF. Extensive portions of the seafloor consist of mantle rocks, and the microbial communities they hold may be important mediators for geochemical and energetic processes in the subsurface [9]. Comprehensive sampling of a few key targets was prioritized over a survey of many locations, allowing for a multidisciplinary dataset, including the metagenomic analyses presented here [7].

The LCHF is unusual in that it is driven by serpentinization ( $Olivine/Pyroxene + H_2O \rightarrow Serpentine + H_2 + CH_4 + \Delta$ ) rather than magmatic activity. This geochemical process yields products such as hydrogen gas and methane which can be utilized by potential primary producers at the Lost City, allowing this microbially diverse ecosystem to proliferate. Hydrogen gas is released as a result of the hydration and oxidation of iron minerals, making it a key component in the biological significance of serpentinization [9]. Hydrogen is an integral reductant amongst subsurface microbial communities, and there exists a distinct, circulating

**Table 1** Mean values for physical properties measured at LCHF markers (nd = no data) [39].

Location	T <sub>max</sub> (°C)	pH at T <sub>max</sub>	SO <sub>4</sub> <sup>2-</sup> (mmol/kg)	H <sub>2</sub> S (mmol/kg)	H <sub>2</sub> (mmol/kg)	CH <sub>4</sub> (mmol/kg)
CALY	29.8	9.4	17.1	0.9	nd	nd
PoNS	48.2	9.1	15.9	0.6	1.2±0.1	0.2±0.0
PoCH	85.0	8.9	10.9	0.2	2.0±0.1	0.2±0.0
SOM	58.4	8.3	12.9	0.2	0.7±0.5	0.1±0.1
IMAX	61.6	9.0	7.4	2.2	2.8±0.7	1.1±0.3

system feeding the LCHF [7]. Temperatures at the LCHF do not exceed ~110°C, preventing the removal of sulfate as anhydrate due to insufficiently high temperatures [9]. Thus, sulfate is an abundant electron acceptor at the Lost City, and it is cycled throughout the chimneys and utilized by associated microbial communities.

One candidate for a chemoautotrophic organism that may be metabolizing hydrogen gas and sulfate is UBA665 spp., a member of the order Thermodesulfobivibrionales, and represented by our new metagenome-assembled genomes (MAGs) CALYbin001 and SOM1bin045. Our data suggests that this organism is metabolically flexible and may be a fundamental member of this hydrothermal ecosystem.

## Methods

### Sample collection

Samples were collected during the AT42-01 cruise by the submersible ROV Jason, which was equipped with the HOG sampler, designed to collect hydrothermal fluids for biogeochemical and microbiological analyses [18]. Hydrothermal fluid samples were collected from multiple locations across the LCHF, as shown in Figure 1.

### DNA extraction and sequencing

Extraction of DNA from cells retained on the 0.2- $\mu$ m Sterivex filters was performed according to an established laboratory protocol [20, 33]. Metagenomic libraries were prepared and sequenced as described by Thornton et al. [20]. Quality control and sequencing of the metagenomic libraries was conducted at the University of Utah High-Throughput Genomics Core Facility. Paired-end sequencing (2  $\times$  125 bp)

was performed on an Illumina HiSeq2500 platform with HiSeq v4 chemistry.

### Metagenomic analysis

Quality-filtered and trimmed reads from all libraries were co-assembled using Megahit v1.1.1 [32] to produce a single assembly representing all fluid samples. In addition, chimney-specific assemblies were conducted for CALY, IMAX, PoCH, SOM1, and SOM2 using metaSPAdes v3.13.0 [21] as implemented by the KBase platform (kb\_SPAdes v.1.2.4) [22]. A chimney-specific assembly from the reads obtained from PoNS was also performed with Megahit v1.1.1, as with the pooled assembly. Binning of MAGs from the pooled Megahit assembly and PoNS Megahit assembly was conducted with BinSanity using the Binsanity-lc workflow v0.2.6.2 [23]. Binning of MAGs from the chimney-specific metaSPAdes assemblies on KBase was conducted with MaxBin2 v2.2.4 (kb\_maxbin v.1.1.1) [24], MetaBAT2 v2.2 (kb\_metabat v.2.3.0) [25], and DAS Tool v1.1.2 (kb\_das\_tool v.1.0.6) [34]. MAGs were assigned taxonomic classifications with GTDB-Tk classify (kb\_gtdbtk v.0.1.4) [26]. Predicted proteins encoded by MAGs were annotated by GhostKOALA v2.2 [1], and the protein identifications reported here were supplemented by annotations from InterProScan 5 (v5.52-86.0) [27]. Completeness and redundancy of MAGs were calculated with CheckM v1.0.5 [28].

The coverage of each MAG was calculated as the sum of the normalized coverages of its member contigs, where the coverage of each contig was calculated as the total sequencing coverage of reads mapped to the contig by bowtie2 [17] (as measured by the genomecov command in bedtools [29]),

divided by the length of the contig. Contig coverages were normalized to library size by dividing the coverage by the total mapped read coverage for that library. Normalized coverages were multiplied by  $10^6$  for final units of fragments (or transcripts) per million (TPM).

### Phylogenetic analysis

Phylogenetic trees of the 16S rRNA gene amplicon sequences were constructed with RAxML [35] as implemented by the SILVA web server [36]. Percent identity between genomes was calculated with Clustal Omega [19].

## Results

### Thermodesulfovibrionales MAGs

Shotgun metagenomic sequences were obtained from hydrothermal fluids collected during the 2018 expedition to the Lost City hydrothermal field. Thermodesulfovibrionales as represented by two bins, SOM1bin045 and CALYbin001, appear to be most abundant at the Poseidon North Spire (PoNS) and Calypso (CALY) locations (Table 2). Abundant organisms are likely utilizing sulfate as an electron acceptor, with a variety of potential electron donors available from the environment. Completeness scores of CALYbin001 and SOM1bin045 are 80.85% and 84.50%, respectively, and both bins are estimated to contain <1% contamination.

### Sulfur metabolism

Genes encoding for the full dissimilatory sulfate reduction and oxidation pathway [1], most notably the DsrAB complex (gene ID 1349.14,15 in CALYbin001; gene ID 3213.13,14 in SOM1bin045) (Table S1), are present in Lost City Thermodesulfovibrionales, suggesting full reduction of sulfate to sulfide. The assimilatory sulfate reduction pathway [1] is largely incomplete, with the exception of Sat (622.15, 5401.09), which is redundant to the dissimilatory pathway. DsrC is encoded by both bins (1349.09; 3213.09), potentially functioning as a redox hub in dissimilatory sulfur reduction [15]. No SOX genes are present; sulfate is likely the primary oxidant utilized by this organism. InterProScan results suggest that sulfate is likely transported into the

cell via ABC superfamily transporters, specifically modB (6250.07), which is a molybdenum transporter that has been shown to transport molecules of similar conformation such as sulfate [16]. The QmoABC complex (622.20,21,22; 5401.02,03,04) and a portion of the DsrMKJOP complex (1349.07; 3213.07), which both connect the Q-Pool to sulfur reduction [37, 38], appear to be present as well. QmoABC appears to be necessary for sulfate reduction in *Desulfovibrio vulgaris*, but not sulfite reduction [37].

### Nitrogen

CALYbin001 encodes a complete NifDHK complex (1700.10,11,15-18) [1], allowing the potential for nitrogen fixation. SOM1bin045 lacks any specific subunits of this complex as defined by KEGG or InterProScan, though InterProScan results for SOM1bin045 detect a nitrogenase molybdenum iron protein domain (1366.26, 33184.02), which facilitates binding of  $N_2$  [14].

### Hydrogen

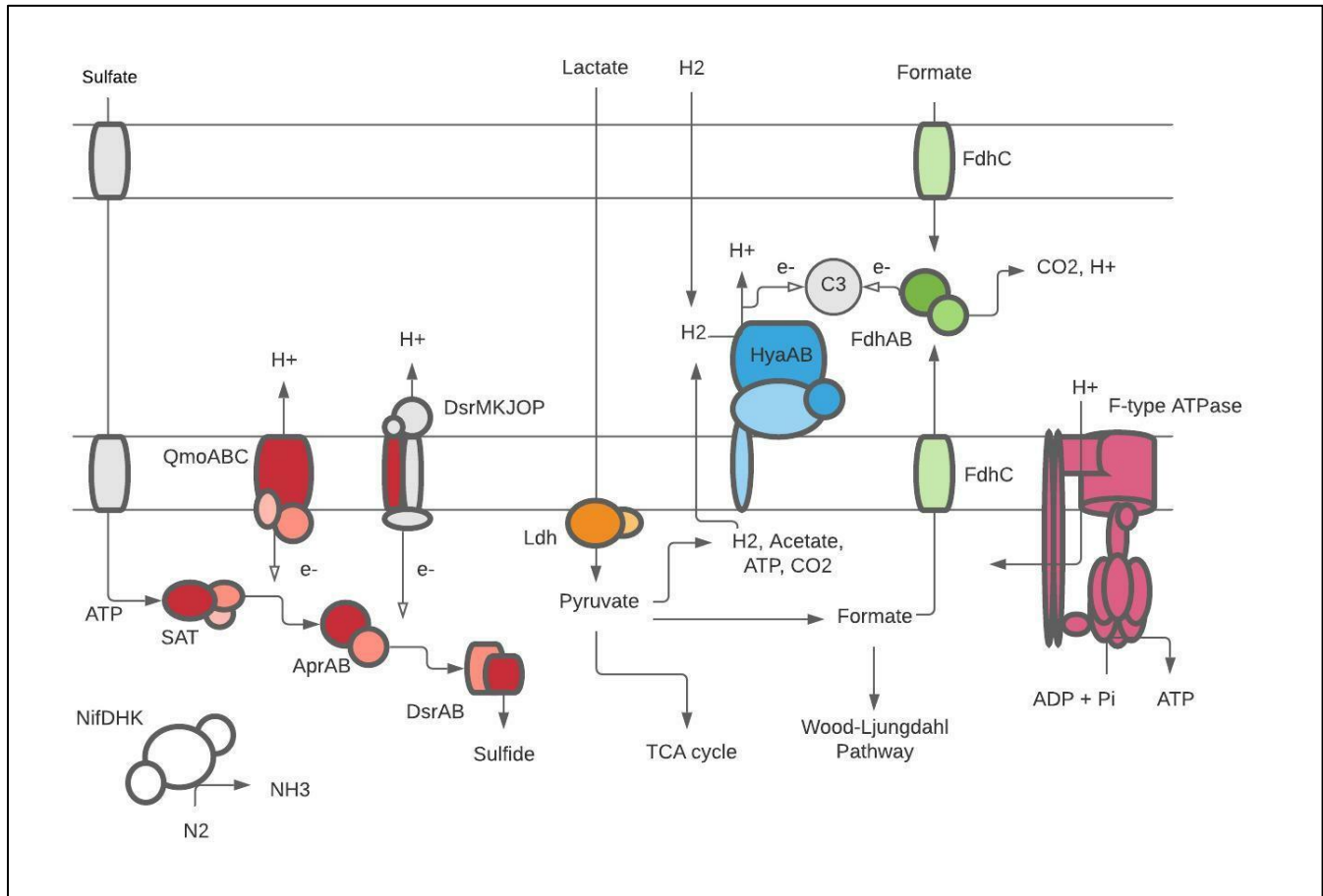
Multiple hydrogenases are present, including the [NiFe]-hydrogenase Hyd-1 (Group 1) large and small subunits (1030.15,18; 1588.07,10), a NADP-reducing hydrogenase (9861.07,09), and a [4Fe-4S] cluster membrane-bound hydrogenase (10750.02): HyaAB, HndC, and MbhJ, respectively. Group 1 hydrogenases include membrane-bound respiratory uptake hydrogenases which couple  $H_2$  oxidation to a cytochrome, resulting in proton pumping across the membrane [3, 4]. Additionally, Group 1 hydrogenases can recapture  $H_2$  produced by nitrogenases [2]. Hyd-1 hydrogenases are believed to have a role in hydrogen cycling during fermentative growth [40]. NADP-reducing hydrogenases such as HndC catalyze the reduction of NADP in the presence of molecular  $H_2$  to yield NADPH. MbhJ is a subunit of a hydrogen-evolving hydrogenase that utilizes protons both as a substrate for hydrogen production and proton translocation [6].

### Carbon

Multiple formate dehydrogenases (FdhAB, FdhC, FdhD) are present. FdhAB (5883.05; 24862.05) catalyzes the reversible transformation between

**Table 2** Normalized coverage (in TPM) of LC *Thermodesulfobibrionales* (UBA665 spp.) bins across sampling sites.

	CALY	PoNS	PoCH	SOM1	SOM2	IMAX
CALYbin001	154591	131937	156	5698	1315	5230
SOM1bin045	178094	83900	111	1457	995	6397



**Figure 2** Metabolic pathways inferred from the predicted protein sequences and functions encoded by the *Thermodesulfobibrionales* (UBA665 spp.) MAG. Proteins relevant to sulfur reduction are highlighted in red and include the SAT (K00958), AprAB (K00394, K00395), DsrAB (K11180, K11181), QmoABC (K16885, K16886, K16887), and DsrMKJOP (K00374) complexes. Proteins involved in formate transport and reduction are highlighted in green and include the FdhC transporter (K21993) and FdhAB complex (K05299). The HyaAB complex (K06282, K06281) facilitates the oxidation of hydrogen gas (H<sub>2</sub>). Lactate dehydrogenase (Ldh) (K00016) allows for the catabolism of lactate to pyruvate. Ambiguous proteins (either presence is unknown, or there are multiple types that may be filling this role) are highlighted in gray, and complexes only present in one of the two bins are shown but not highlighted. Solid arrows represent movement of molecules and protons; empty arrows represent movement of electrons.

formate and carbon dioxide [6]. FdhC (1349.03; 3213.03) is a formate transporter, and FdhD (21896.01; 27133.02) is a sulfur carrier. LC *Thermodesulfobibrionales* encode lactate dehydrogenase (Ldh) (27102.01; 52498.01), which is able to catalyze the conversion of lactate to pyruvate [6]. Pyruvate can be converted into acetate, CO<sub>2</sub>, H<sub>2</sub>, and ATP in the presence of ferredoxin, a process which may be facilitated by encoded pyruvate dehydrogenases (411.14; 661.15). Pyruvate has the potential to undergo a variety of alternative reactions, yielding formate

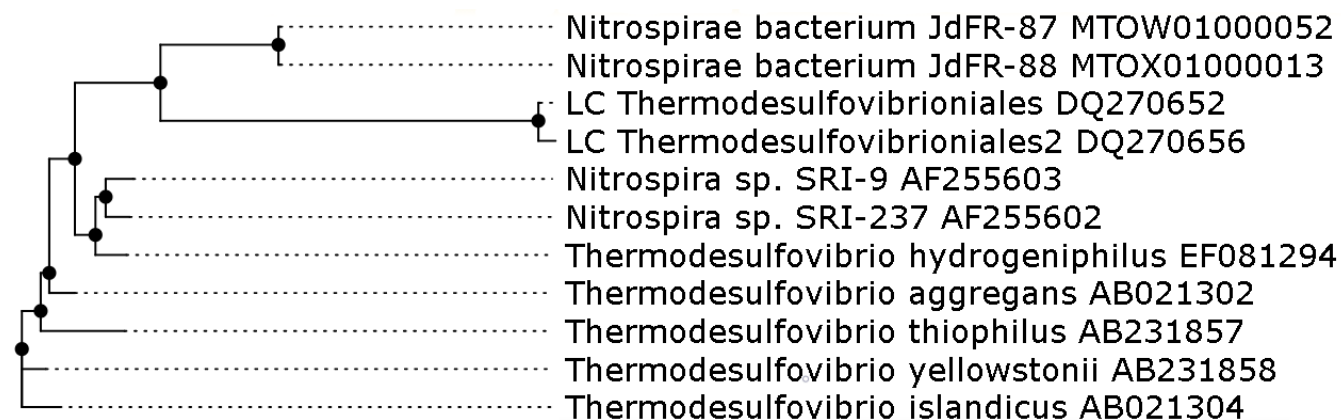
through a pyruvate formate-lyase-activating enzyme (3007.07, 1530.02; 8451.06, 46260.05, 9709.06), acetyl-CoA via the PorABCD complex and KorB (7086.04,05,06, 6734.04; 55233.02, 20973.03,04,05, 24873.04), or oxaloacetate via OadA or Pyc (1035.01,02, 13960.02; 6771.09).

UBA665 spp. possesses most of the TCA cycle as defined by KEGG, and any enzymes that facilitate the missing reaction steps are present in InterProScan results. Therefore, this organism appears to encode all proteins necessary to

complete the full TCA cycle (1035.08,09,11,13,15, 2662.05, 2985.09, 13670.01, 622.09, 1739.12). Additionally, through similar analysis, it is suggested that UBA665 spp. encodes a complete Wood-Ljungdahl pathway (30797.04, 1632.05, 17107.03, 5883.05,09 14926.15, 7086.03; 24873.05, 24862.03,04,05, 44068.02, 55233.02, 22072.04, 20973.02, 9861.11). Five enzymes from the Calvin cycle are present (2848.07,08, 411.14,15, 1632.12, 1280.17; 3764.12,13, 661.14,15, 3512.09, 1246.22), but this cycle is largely incomplete.

### Phylogeny

16S Illumina sequences obtained for CALYbin001 and SOM1bin045 register 99.6% identity to one another. Percent identity between LC Thermodesulfovibrionales 16S rRNA and the closest neighboring branches defined by SILVA is  $\sim 84.0 \pm 0.2\%$ .



**Figure 3** Unrooted phylogenetic tree representing LC Thermodesulfovibrionales and neighbors identified by SILVA. Percent identity between LC Thermodesulfovibrionales 16S rRNA and the closest adjacent branches is  $\sim 84 \pm 0.2\%$ .

Other taxonomic classifications for Thermodesulfovibrionales species have been proposed, and the NCBI database provides an alternative classification for UBA665 spp. that lacks the specificity provided by GTDB-Tk classify ( $\sim 79.0 \pm 2.0\%$  amino acid identity between LC Thermodesulfovibrionales and the genus UBA665). NCBI classifies this organism under the order and family Nitrospirales Nitrospiraceae, rather than Thermodesulfovibrionales UBA9935 (GTDB-Tk) [10].

### Discussion

Thermodesulfovibrionales found at the LCHF (UBA665 spp.) appear to be metabolically flexible. LC Thermodesulfovibrionales are likely able to grow on environmental sulfate, lactate, hydrogen, and formate, depending on availability. The presence of multiple hydrogenases in SOM1bin045 suggests variable use of  $H_2$ .

In the presence of methanogens such as *Methanothermobacter thermoautotrophicus*, strains of *Thermodesulfovibrio islandicus* and *Thermodesulfovibrio aggregans* are able to fully metabolize lactate more quickly [30]. One study demonstrated that strains of *T. islandicus* and *Thermodesulfovibrio hveragerdense* isolated from an Icelandic hot spring can grow on lactate, pyruvate, and  $H_2$ . In this same study, *T. islandicus* could additionally utilize formate and reduce nitrate [31]. *Thermodesulfovibrio hydrogeniphilus* isolated from a Tunisian hot spring could readily

grow on  $H_2$  but could only grow on formate poorly [5]. Therefore, it is likely that LC Thermodesulfovibrionales utilizes  $H_2$  as a primary electron donor, with formate as a viable secondary donor, and possesses the ability to metabolize lactate when in association with biofilms or when in close proximity to syntrophic partners. It is unknown whether the main source of  $H_2$  is environmental, or a byproduct of other metabolic pathways encoded by this or other organisms, and it may be variable dependent on location within the chimney and the stage of UBA665 spp.'s reproductive cycle.



The presence of enzymes encoding a complete TCA cycle and Wood-Ljungdahl pathway also suggest that UBA665 spp. possesses the capacity for heterotrophy and autotrophy. Utilization of the Wood-Ljungdahl pathway in the subsurface and carrying out the TCA cycle when in association with chimney biofilms is likely to be advantageous in this setting.

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### **Supplementary Information**

Table S1,

<https://doi.org/10.6084/m9.figshare.15025431>