Metagenomic Comparison of Two *Thiomicrospira* Lineages Inhabiting Contrasting Deep-Sea Hydrothermal Environments

William J. Brazelton*, John A. Baross

School of Oceanography and Center for Astrobiology and Early Evolution, University of Washington, Seattle, Washington, United States of America

**Abstract**

**Background:** The most widespread bacteria in oxic zones of carbonate chimneys at the serpentine-hosted Lost City hydrothermal field, Mid-Atlantic Ridge, belong to the *Thiomicrospira* group of sulfur-oxidizing chemolithoautotrophs. It is unclear why *Thiomicrospira*-like organisms thrive in these chimneys considering that Lost City hydrothermal fluids are notably lacking in hydrogen sulfide and carbon dioxide.

**Methodology/Principal Findings:** Here we describe metagenomic sequences obtained from a Lost City carbonate chimney that are highly similar to the genome of *Thiomicrospira crunogena* XCL-2, an isolate from a basalt-hosted hydrothermal vent in the Pacific Ocean. Even though *T. crunogena* and Lost City *Thiomicrospira* inhabit different types of hydrothermal systems in different oceans, their genomic contents are highly similar. For example, sequences encoding the sulfur oxidation and carbon fixation pathways (including a carbon concentration mechanism) of *T. crunogena* are also present in the Lost City metagenome. Comparative genomic analyses also revealed substantial genomic changes that must have occurred since the divergence of the two lineages, including large genomic rearrangements, gene fusion events, a prophage insertion, and transposase activity.

**Conclusions/Significance:** Our results show significant genomic similarity between *Thiomicrospira* organisms inhabiting different kinds of hydrothermal systems in different oceans, suggesting that these organisms are widespread and highly adaptable. These data also indicate genomic processes potentially associated with the adaptation of these lineages into strikingly different habitats.

**Introduction**

Microbial oxidation of sulfur is the basis of most ecosystems at seafloor hydrothermal environments. In basalt-hosted hydrothermal vents, hydrogen sulfide (H$_2$S) is the most abundant electron donor driving primary production [1]. Concentrations of H$_2$S are ~3 mmol/kg in typical basalt-hosted systems and can reach >40 mmol/kg at some sites [2]. Much of the animal biomass in these systems is directly supported by symbiotic H$_2$S-oxidizing bacteria. At the serpentine-hosted Lost City hydrothermal field in the Atlantic Ocean, H$_2$S concentrations are lower [2], ranging between 0.05–2.8 mmol/kg in end-member hydrothermal fluids [3]. Nevertheless, mussels collected from Lost City chimneys harbor endosymbionts with close phylogenetic relationships to H$_2$S-oxidizing as well as methane-oxidizing bacteria [4]. Megafaunal biomass is much lower at Lost City compared to most basalt-hosted systems, however, and the relative lack of H$_2$S is the most likely cause [2]. Because fluid chemistry at Lost City is dominated by subsurface serpentinization reactions, hydrogen (H$_2$, 1-15 mmol/kg) and methane (CH$_4$, 1-2 mmol/kg) are much more abundant in chimney fluids than H$_2$S [2]. Accordingly, archaea related to methanogens and methanotrophs comprise >80% of all detectable cells in biofilms associated with the hot, anoxic interiors of actively venting chimneys [5].

Bacteria are more abundant in biofilms attached to the outer walls of Lost City chimneys where hydrothermal fluids mix with cold, oxygenated seawater [3]. The most commonly detected bacteria in Lost City chimneys and fluids belong to the *Thiomicrospira* genus of *Gammaproteobacteria* [6,7]. *Thiomicrospira* species frequently inhabit zones of hydrothermal chimneys and sediments where H$_2$S and oxygen are both present [8,9]. The basalt-hosted hydrothermal systems in which *Thiomicrospira* species are typically found are characterized by acidic fluids that contain abundant H$_2$S and carbon dioxide (CO$_2$). In contrast, the fluids exiting from Lost City chimneys are alkaline (pH 9–11), contain only moderate amounts of H$_2$S, and are nearly devoid of CO$_2$ [2,10]. Nevertheless, the most prevalent bacterial 16S rRNA sequences in these fluids are affiliated with genus *Thiomicrospira* [6]. No Lost City *Thiomicrospira* have yet been cultivated, and it is unknown how they have adapted to these extreme environmental...
isolated from basalt-hosted systems with H2S concentrations and high pH of Lost City fluids, however.

Here we compare metagenomic data from a Lost City carbonate chimney containing a large number of Thiomicrospira-related sequences to the only completed genome sequence of a Thiomicrospira organism: *Thiomicrospira crunogena* XCL-2 [12], which was isolated from diffuse fluids at the Galapagos Rift [13], a basalt-hosted hydrothermal system in the Pacific Ocean. H2S concentrations (~0.2 mmol/kg) in fluids venting from surface sediments at the Galapagos Rift are diluted due to extensive mixing with seawater, and H2S levels in subsurface sediments are estimated to be much greater [14]. Additional strains with nearly identical 16S rRNA sequences to that of *T. crunogena* XCL-2 [15] have been isolated from basalt-hosted systems with H2S concentrations around 3–7 nmol/kg [16]. In contrast, H2S in Lost City end-member fluids never exceeds 2.8 mmol/kg and is much lower within carbonate chimneys where end-member fluids mix with ambient seawater [3]. Lost City fluids also have higher pH and lower CO2 concentrations than these basalt-hosted systems [16,2]. Thus the metagenomic dataset described here provides a revealing snapshot of genomic changes associated with the divergence of two lineages into geochimically distinct habitats.

**Methods**

**DNA extraction**

The data described here are a subset of the dataset first reported in [17]. The carbonate chimney sample (H03_072705_R0424) was collected from the central ‘Poseidon’ edifice of the Lost City Hydrothermal Field (depth, 735 m; latitude, 30.12; longitude, –42.12) on 27 July 2005 by the DSV *Hercules* during the 2005 Lost City Expedition aboard the R/V *Ronald H. Brown*. Chimney material was frozen at ~80°C immediately after collection and remained frozen until onshore analysis. DNA was extracted according to a protocol modified from previous reports [6,18] and summarized here. After crushing a frozen carbonate sample with a sterile mortar and pestle, approximately 0.25–0.5 g of chimney material was placed in a 2 mL microcentrifuge tube containing 250 μL of 2X buffer AE (200 mM Tris, 50 mM EDTA, 200 mM NaCl, pH 8) and 2 μg of poly-dIdC (Sigma-Aldrich) and incubated at 4°C overnight to allow chelation of salts and binding of DNA to poly-dIdC. Between 36–72 replicate tubes were processed in parallel, and a total of ~1 kg of carbonate minerals were processed. This protocol involved no size fractionation between sample collection and DNA extraction. Proteinase K (final concentration 1.2 mg/mL) and 10 μL of 20% SDS were added to each tube before incubation at 37°C for at most 30 min. A further 150 μL of 20% SDS and 500 μL of phenol:chloroform:miscosoymal alcohol (25:24:1 ratio by volume) were added to each tube before centrifugation at 12,000 g for 10 min. Supernatants were transferred to clean tubes for a second phenol:chloroform:miscosoymal alcohol extraction. After centrifugation, supernatants were pooled into SnakeSkin dialysis tubing (Pierce Protein Research Products; Rockford, IL) and dialyzed against 200 mM EGTA overnight at 4°C. This large scale dialysis step proved to be very efficient in removing minerals and organic inhibitors. After dialysis, DNA was precipitated by adding 0.1 vol 3 M sodium acetate and 1 vol isopropanol and stored at ~20°C for 2–4 hours. Pellets were collected by centrifugation at 16,000 g for 20 min at 8°C, washed once in 70% ethanol, dried in a vacuum centrifuge, and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8).

**Metagenomic sequencing and annotation**

Library construction and Sanger end-sequencing of pUC18 inserts was conducted according to the standard protocols at the DOE Joint Genome Institute in 2007. Two libraries were constructed from two subsamples of the same carbonate chimney sample. Reads from both libraries were combined for assembly and for analyses described here. Mean read length for the 46 361 reads from both libraries was 755 bp, and the mean length of all 6324 contigs was 1583 bp. All sequencing reads are deposited under GenBank accession numbers ACQI01006325–ACQI01026573, and assembled contigs are deposited under accession numbers ACQI01000001–ACQI01006324. End-paired sequences are those with identical names and different suffix: eg. FNHG1000.b1 and FNHG1000.g1; FOSS3464.x1 and FOSS3464.y1. Open reading frames were assigned with Glimmer [19] and compared with *T. crunogena* genes by blastp [20]. Potential homologs for all *T. crunogena* proteins were identified by searching against a database of all Lost City metagenomic contigs or all unassembled sequencing reads with blastn [20]. Visualization of BLAST results was facilitated by use of Artemis [21]. An Artemis file containing annotated open reading frames for Lost City metagenomic contigs is available at http://www.staff.washington.edu/braz. Files enabling identification of paired end sequences and membership of sequences in contigs are also available at the above website.

**Results and Discussion**

**Comparison of genomic content**

As previously reported [17], we obtained 35 Mb of metagenomic sequence from 46 361 shotgun reads of two pUC18 libraries constructed by the DOE Joint Genome Institute with DNA extracted from ~1 kg of a single Lost City carbonate chimney sample. A large proportion of the total shotgun reads (14.6%) had BLASTN alignments >500 bp with the *Thiomicrospira crunogena* XCL-2 genome [12]. Most of these reads exhibited 67–71% nucleotide similarity with *T. crunogena*, and very few reads contained sequences with >84% similarity (Figure 1). These data indicate that the Lost City metagenomic dataset is dominated by a population that is genotypically uniform and contains moderate sequence similarity with *T. crunogena*.

Approximately half of the shotgun reads assembled into 6324 contigs, including 49 contigs >7 kb in length. Almost all of the large contigs contained open reading frames with significant sequence similarity to a *Thiomicrospira crunogena* XCL-2 protein, indicating that *Thiomicrospira*-related sequences comprise a high proportion of the metagenomic assembly (Figure 2). The similar sequencing coverage of the largest contigs is consistent with a single population dominating the dataset (Figure 2A). These large contigs are ~38%GC (Figure 2B), and the *T. crunogena* genome is 43%GC.

Our previous work has shown that multiple *Thiomicrospira* taxonomic units are present in Lost City chimneys, but the local conditions at each chimney determine which sequences are the most abundant [6]. The 16S rRNA sequence which dominates the youngest, warmest chimneys matches that in Lost City metagenomic contig C2148 (Figure 3). Nearly half of all bacterial 16S rRNA clones in a library constructed from the same DNA preparation used for metagenomic sequencing showed high similarity to *T. crunogena* (data reported in [17]), and half of these clones were nearly identical to the sequence in contig C2148.
Therefore, the largest metagenomic contigs in this study are likely to represent a *Thiomicrospira* population that is abundant in the youngest, warmest Lost City carbonate chimneys. Although some Lost City chimneys vent fluids containing up to 2.8 mmol/kg H₂S, the sample used in this metagenomic study was collected from a chimney venting 0.3 mmol/kg H₂S (D. Butterfield, manuscript in prep.).

The large contigs contained surprisingly few archaeal sequences considering the dominance of archaea in actively venting Lost City chimneys [5]. Bacteria are known to be more abundant in the exterior, oxygenated zones of the chimneys [5], so the relative lack of archaeal sequences can be attributed to the sample containing little material from interior, anoxic zones of the chimney. These micro-scale redox zones are difficult to identify by bulk mineralogy due to pervasive mixing of ambient seawater through the highly porous carbonate chimneys [22].

Many genes in the *T. crunogena* XCL-2 genome [12] have putative orthologs in the Lost City metagenome; a complete list is available in the Supplementary Information as Table S1. Of the 2200 *T. crunogena* protein-coding genes (obtained from the Joint Genome Institute IMG database), 652 had tblastn [20] hits with E values better than 10⁻²¹⁰ to a Lost City contig or unassembled sequence (1217 hits better than 10⁻²⁵⁰; 1842 hits better than 10⁻¹⁰). Of the 358 *T. crunogena* proteins lacking hits with E values better than 10⁻¹⁰, 214 were annotated as hypothetical proteins. Gene order is also highly conserved between the Lost City contigs and the *T. crunogena* genome, as exemplified by the largest contig (Figure 4). It is clear that large genomic rearrangements have occurred since the divergence of the two lineages, however, as the Lost City contig is syntenic with two distinct *T. crunogena* regions separated by 161 kb (Figure 4). Interestingly, three of the open reading frames (ORFs) in this contig encode proteins that are more similar to other bacteria (*Methylophaga*, *Marinobacter*) that have been identified in Lost City chimneys and fluids [6] and are in reverse orientation with respect to surrounding ORFs. Assembly error is an unlikely explanation for this result because in each case 3–6 sequencing reads contained at least one of these three ORFs as well as a nearby ORF with high sequence similarity to *T. crunogena*. These observations are consistent with an origin of these ORFs by lateral gene transfer, but further sequencing and phylogenetic analyses are required to test this hypothesis.

Another striking genomic difference between *T. crunogena* and Lost City metagenomic sequences is the presence of a prophage genome in *T. crunogena* but not in the corresponding Lost City contig (Figure 5). Genes flanking both ends of the *T. crunogena* prophage are present in the same Lost City contig, but no prophage sequences are present. Furthermore, a possible direct repeat caused by the insertion of the prophage genome into *T. crunogena* is also absent from the Lost City contig (Figure 5). If the prophage was previously present in the Lost City sequence and subsequently lost, the direct repeat is expected to have remained. Therefore, the absence of the prophage and direct repeat in the Lost City contig indicates that the prophage was inserted into *T. crunogena* after its divergence from the Lost City lineage.

Figure 1. Most metagenomic reads from a Lost City carbonate chimney have moderate sequence similarity with *Thiomicrospira crunogena* XCL-2. Shotgun reads with >500 bp BLASTN alignments with the *T. crunogena* genome are binned according to the nucleotide sequence similarity (x-axis) of the BLASTN alignments. The y-axis represents the *T. crunogena* coverage for the reads in each bin where coverage corresponds to the ratio of the total number of aligned base pairs in each bin to the total number of base pairs in the *T. crunogena* genome, as in Figure 2 of [31]. doi:10.1371/journal.pone.0013530.g001

Figure 2. The Lost City *Thiomicrospira* population is represented by the largest metagenomic contigs. (A) All contigs larger than 10 kb have tblastn hits with at least one *T. crunogena* protein with an E value better than 10⁻³⁰. These large contigs are present at 5–8x sequencing coverage. (B) Large contigs with *T. crunogena*-related sequences are ∼38%GC; the *T. crunogena* genome is 43%GC. doi:10.1371/journal.pone.0013530.g002
Comparison of sulfur utilization genes

The data presented thus far indicate that the Lost City Thiomicrospira population and T. crunogena represent two moderately closely related, but clearly distinct, lineages. We further examined the Lost City metagenomic data to examine whether these two lineages inhabiting contrasting hydrothermal systems contain similar sulfur utilization genes. T. crunogena utilizes the Sox pathway for complete oxidation of various sulfur compounds to sulfate [12]. We identified apparent homologs for all Sox genes required for sulfite-, thiosulfate-, elemental sulfur (S0), and H2S-dependent cytochrome c reduction in Lost City metagenomic contigs (Figure 6). Amino acid similarities between putative homologs range between 61% and 89%, and gene order appears to be conserved (Figure 6). Interestingly, soxB and soxC are not contiguous with soxXYZA in both the T. crunogena genome and the Lost City metagenomic contigs. It is unclear whether this genomic arrangement has been maintained in both lineages by selection or if it is a result of recent evolutionary divergence.

In most organisms utilizing the Sox pathway, all genes are organized into a single cluster, and the few exceptions may have arisen as a result of lateral gene transfer [23]. Phylogenetic discrepancies between T. crunogena SoxC (and the highly similar Lost City SoxC) compared to the other Sox proteins are consistent with one or more lateral gene transfer events [23]. Considering this phylogenetic interpretation, the presence of a transposase near the Lost City soxCD sequences (Figure 6) is intriguing, and further work should investigate whether transfer of soxC is mediated by transposases in Lost City chimneys. Scott et al. [12] have also suggested that the fragmented arrangement of Sox genes in T. crunogena could be maintained by the lack of selection pressure for regulation of a constitutively expressed pathway. Thus the Sox genes in the Lost City metagenome may be constitutively expressed, and at least some of them were likely acquired by an ancestor common to T. crunogena via lateral transfer.

Additional details of sulfur utilization by Lost City Thiomicrospira organisms can be inferred from the metagenomic data. One Lost City unassembled read (GenBank ACQI01023028) contains an open reading frame with 81% amino acid identities to a putative sulfide:quinone reductase (SQR) encoded by T. crunogena gene Tcr_1170. In Rhodobacter capsulatus, SQR is known to catalyze the reduction of H2S to S0, which accumulates as S0 granules outside the cells, and S0 is also deposited extracellularly by T. crunogena under certain conditions [24]. Alternatively, sulfur deposition in T. crunogena could be the result of ineffectual interactions among SoxC, SoxYZ and SoxB due to their differing phylogenies [23].
Intracellular $S^0$ granules have been observed in unidentified filamentous microorganisms collected from Lost City chimneys [25], but extracellular $S^0$ granules have not yet been reported. The *T. crunogena* genome is also notable for its lack of genes encoding sulfate assimilation enzymes, indicating that it depends entirely on reduced sulfur species. This also appears to be true for the Lost City *Thiomicrospira* representative, as none of the metagenomic contigs with *Thiomicrospira*-related sequences contain ATP sulfurylase, APS kinase, or PAPS reductase. Apparent homologs for the latter two sequences were identified in very short contigs, but the taxonomic affiliation of these contigs is not readily apparent.

**Comparison of carbon fixation genes**

Considering the very low CO$_2$ concentrations in Lost City chimney fluids [2,10], it is expected that Lost City *Thiomicrospira* organisms harbor adaptations for living in a low CO$_2$ environment. Indeed, we identified metagenomic sequences encoding a partial

---

**Thiomicrospira crunogena XCL-2 genome**

![Diagram](image)

Lost City carbonate chimney metagenomic contig C5441

---

**Figure 5.** The proteome genome identified in *T. crunogena* is not present in the Lost City metagenome. ORFs on both sides of the proteome are present in Lost City metagenomic contig C5441 (accession # ACQI01005441), but a possible direct repeat (upstream region has 88% identities with positions 24–49 of Tcr_0702) is absent. Amino acid identities and alignment lengths (as calculated by blastp) are shown. doi:10.1371/journal.pone.0013530.g005

The genome of *T. crunogena* encodes three different RubisCO enzymes, two form I RubisCOs and one form II Rubisco [12]. The form II Rubisco is only expressed under high CO$_2$ concentrations [27]. The Lost City metagenomic data includes apparent homologs for the two form I Rubisco genes. (Two unassembled sequences encode form II RubisCOs, but their closest relatives do not include *T. crunogena*). Although no firm conclusions can be drawn from the absence of genes in an incomplete metagenomic dataset, it appears that the low CO$_2$ concentrations in Lost City fluids have rendered the high-CO$_2$ form II Rubisco unnecessary for Lost City *Thiomicrospira*.

**Comparison of transposase sequences**

We have previously observed that the Lost City metagenome contains a surprisingly high abundance and diversity of sequences

---

**Thiomicrospira crunogena XCL-2 genome**

![Diagram](image)

Lost City carbonate chimney metagenomic contig C5441

---

**Figure 7.** Most of the genes in the carboxysome operon of *Thiomicrospira crunogena* are present in Lost City metagenomic sequences. Amino acid percent identities between putative homologs are shown. The Lost City sequences are incomplete; it is expected that further sequencing and assembly will yield a complete carboxysome operon, cbbL and cbbS encode the large and small subunits of RubisCO, csoS3 encodes carbonic anhydrase. All other genes are expected to encode structural proteins forming the carboxysome shell. Accession numbers for contigs C1529 and C1530 are ACQI01001529 and ACQI01001530. doi:10.1371/journal.pone.0013530.g007
that encode transposases [17]. The transposase sequences were found in all of the smallest, highest coverage contigs and were rarely found in large contigs, suggesting an origin from viruses or extragenomic molecules. Another possibility is that these contigs represent genomic regions that are not amenable to assembly into larger contigs. Genomic regions containing transposase sequences can be expected to be highly variable, and such non-consensus sequences could not be assembled into large contigs with the sequencing effort of this study. Therefore, it is plausible that many of the transposase-containing contigs identified in our earlier report [17] represent variable regions of the Lost City Thiomicrospira pangenome, which differ among closely related strains within the Lost City Thiomicrospira phylotype.

To test this hypothesis, we examined whether the largest Thiomicrospira-like contigs are physically linked with unassembled transposase sequences; i.e. we searched for paired end sequences of the cloned metagenomic fragments where one member of the pair was assembled into a large contig and one member contained a transposase sequence. Figure 8 shows that 16 of the 17 contigs >10 kb contain at least one sequence paired with a transposase-containing sequence. Of the 1294 sequences that comprise these contigs, 49 (3.8%) were paired with a transposase sequence. If we conservatively estimate that a single transposase sequence is associated with each of the 16 contigs (244 kb), then a completed genomic assembly should contain one transposase per 15 kb. In comparison, T. crunogena contains only 20 transposases: 0.8% of the genome, or one per 120 kb (from the annotation at http://img.jgi.doe.gov). Although these results must be considered preliminary until a finished genome sequence is obtained, they are suggestive that the Lost City Thiomicrospira genome has a high transposase content.

This analysis also sheds some light on the nature of the extremely high-coverage, transposase-containing contigs (the top-left cluster in Figure 8). The arrows in Figure 8 indicate that three of these contigs are paired with sequences on three of the large, Thiomicrospira-like contigs. Therefore, these transposases are probably part of the Lost City Thiomicrospira genome, and their extremely high coverage (eg. >30x for contig C5672) indicates that they must be present in multiple copies. These examples are rare, however. Most of the high-coverage, transposase-containing contigs were not physically associated with the large, Thiomicrospira-like contigs, and their origin remains unclear. Future genomic sequencing may prove that these contigs, too, represent genomic regions present as multiple copies, but the present data are also consistent with the previous suggestion that they are derived from viruses or small extragenomic molecules [17].

Ecological and evolutionary implications

Among the 358 T. crunogena genes without close relatives in the Lost City metagenome, a few have apparent ecological implications. For example, the phosphonate operon (encodes the ability to utilize organic phosphate) in T. crunogena is not syntenic with any Lost City contigs, and only some of the genes in the phosphonate operon appear to have homologs in Lost City sequences. In contrast, Lost City contigs contain sequences encoding both the low-affinity and high-affinity (inorganic) phosphate uptake systems present in T. crunogena. Three putative sulfonate transporters in T. crunogena are also missing in the Lost City sequences. Therefore, it is possible that Lost City Thiomicrospira are dependent on inorganic forms of phosphorus and sulfur, although no firm conclusions can be drawn due to the incomplete nature of the metagenome.

Previous studies have noted the inability of T. crunogena to utilize hydrogen gas (H₂) as a sole electron donor despite the presence of a Ni/Fe hydrogenase operon in its genome [12,28]. Considering the abundance of H₂ in Lost City fluids [2], the ability of Lost City Thiomicrospira to utilize H₂ with this hydrogenase operon is of interest. One contig in our dataset (C4437) appears to contain a partial Ni/Fe hydrogenase operon, but these sequences share little similarity with those in T. crunogena and most likely derive from other lineages. Due to the incomplete nature of the metagenomic dataset, however, we cannot conclude with certainty whether Lost City Thiomicrospira harbor hydrogenases.

Considering their apparent inability to utilize the most abundant electron donor (H₂) and their dependence on two substrates (H₂S and CO₂) that are notably lacking in Lost City fluids, it is remarkable that Thiomicrospira are the most widespread and abundant bacteria in Lost City carbonate chimneys [6,7]. The surprising dominance of these organisms is most likely related to patterns of oxygen and CO₂ availability in carbonate chimneys. Those organisms expected to utilize H₂ at Lost City (Methanosarcinales- and Desulfotomaculum-related organisms) are anaerobic and more prevalent in the anoxic, interior zones of carbonate chimneys where the aerobic Thiomicrospira are unlikely to survive [5,6]. Pervasive mixing of oxygenated seawater throughout the highly porous structure of the carbonate chimneys [22] could explain the relative lack of Epislonproteobacteria, as these organisms are typically restricted to areas with low oxygen concentrations [29]. Thiomicrospira, in contrast, can thrive in fully oxic environments as long as reduced sulfur species are present [8]. Furthermore, Thiomicrospira may out-compete Epislonproteobacteria as well as all other bacteria in Lost City fluids because they harbor a carbon-concentrating mechanism that allows them to remain autotrophic at very low CO₂ concentrations (Figure 7). Isotopic analyses indicate that bacteria in Lost City carbonate chimneys are extremely carbon-limited [30], so a carbon-concentration mechanism could be highly advantageous.

Conclusions

In summary, metagenomic analyses of a Lost City carbonate chimney reveal a dominant Thiomicrospira population with similar
Thiomicrospira mussels from Lost City hydrothermal vents. Environ Microbiol 8: 1360–1367.

2. Kelley DS, Korzen JA, Frue-Green GL, Yoeger DR, Shank TM, et al. (2005) A serpentinization-hosted ecosystem: the Lost City hydrothermal field. Science 307: 1428–1434. doi:10.1126/science.1102536.

3. Fruh-Green GL, Bernasconi SM, Butterfield DA, Kelley DS (2004) Sulfur isotope geochemistry of the Lost City hydrothermal vent fluids. Eos Trans AGU 85(48): Abstract B13A-0199.

4. DeChaine EG, Bates AE, Shank TM, Cavanaugh GM (2006) Off-axis axisymmetric found: Characterization and biogeochemistry of bacterial symbionts of Bathymodiolus mussels from Lost City hydrothermal vents. Environ Microbiol 8: 1902–1912. doi:10.1111/j.1462-2920.2005.01115.x.

5. Schrenk MO, Kelley DS, Bolton SA, Baross JA (2004) Low archaeal diversity linked to subsulfur geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. Environ Microbiol 6: 1096–1095.

6. Brazelton WJ, Ludwig KA, Sogin ML, Andreishcheva EN, Kelley DS, et al. (2010) Archaea and bacteria with surprising macrodiversity show shifts in dominance over 1000-year time scales in hydrothermal chimneys. Proc Nat Acad Sci U S A 107: 1612–1617.

7. Brazelton WJ, Schrenk MO, Kelley DS, Baross JA (2006) Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. Appl Environ Microbiol 72: 6257–6270. doi:10.1128/AEM.00574-06.

8. Brinkhoff T, Muyzer G (1997) Increased species diversity and extended habitat range of sulfur-oxidizing Thiomicrospira spp. Appl Environ Microbiol 63: 3798–3796.

9. Jannasch HW, Wiersen CC, Nelson DC, Robertson LA (1985) Thiomicrospira crunogena sp. nov., a Colorless, Sulfur-Oxidizing Bacterium from a Deep-Sea Hydrothermal Vent. Int J Syst Bacteriol 35: 422–424. doi:10.1099/00207713-35-4-422.

10. Proskurinovski G, Lilley MD, Scevel JD, Frueh-Green GL, Olson EJ, et al. (2008) Abiogenic hydrocarbon production at lost city hydrothermal field. Science 319: 605. doi:10.1126/science.1151194.

11. Ruby EG, Jannasch HW (1992) Physiological-Characteristics of Thiomicrospira sp. Strain L-12 Isolated from Deep-Sea Hydrothermal Vents. J Bacteriol 149: 161–163.

12. Scott KM, Sievert SM, Abril FN, Ball LA, Barrett GJ, et al. (2006) The genome of deep-sea vent chemosynthetic bacterium Thiomicrospira crunogena XCL-2. PLoS Biol 4: e303. doi:10.1371/journal.pbio.0040383.

13. Ahmad A, Barry JP, Nelson DC (1999) Phylogenetic Affinity of a Wide, Vacuolate, Nitrate-Accumulating Beggiatoa sp. from Monterey Canyon, California, with Thiomicrospira. Appl Environ Microbiol 65: 270–277.

14. Edmond J, Measures C, Mangum B, Grant B, Takai K (2006) On the formation of metal-rich deposits at ridge crests. Earth Planet Sci Lett 246: 604–607. doi:10.1016/j.epsl.2006.07.003.

15. Wirsen CO, Brinkhoff T, Kuever J, Muyzer G, Molyneaux S, et al. (1998) Comparison of a new Thiomicrospira strain from the Mid-Atlantic Ridge with known hydrothermal vent isolates. Appl Environ Microbiol 64: 4057–4059.

16. Butterfield DA, Seyfried WE, Lilley MD (2003) Composition and evolution of hydrothermal fluids. Energy and Mass Transfer in Marine Hydrothermal Systems. Berlin: Dahlem University Press. pp 127.

17. Brazelton WJ, Baross JA (2009) Abundant transposases encoded by the metagenome of a hydrothermal chimney biofilm. ISME J 3: 1420–1424.

18. Barton HA, Taylor NM, Lubbers BR, Pemberton AC (2006) DNA extraction from low-biomass carbonate rock: An improved method with reduced contamination and the low-biomass contaminant database. J Microbiol Methods 66: 21–31. doi:10.1016/j.mim.2005.10.005.

19. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL (1999) Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27: 4636–4641.

20. Autschel SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402.

21. Rutherford K, Parkhill J, Crook J, Hornell T, Rice P, et al. (2000) Artemis: sequence visualization and annotation. Bioinformatics 16: 944–945.

22. Ludwig KA, Kelley DS, Butterfield DA, Nelson BK, Frueh-Green G (2006) Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. Appl Environ Microbiol 72: 6257–6270. doi:10.1128/AEM.00574-06.

23. Ghosh W, Mallé S, DasGupta SK (2009) Origin of the Sea eukaryote complex system in ancient thermophilic bacteria and coevolution of its constituent proteins. Res Microbiol 160: 409–420. doi:10.1016/j.resmic.2009.07.003.

24. Javor BJ, Wilkot DB, Vetter RD (1998) Phs-Dependent Metabolism of Thiocyanate and Sulfur Globules in the Chemolithotrophic Marine Bacterium Thiomicrospira crunogena. Arch Microbiol 174: 231–238.

25. Dulov L, Lein A, Dubina G, Pimenov N (2005) Microbial processes at the Lost Lake vent field, Mid-Atlantic Ridge. Microb Ecol 47: 97–103. doi:10.1007/s00248-005-0055-6.

26. Dobrinski KP, Boller AJ, Scott KM (2010) Expression and function of four carbonic anhydrase homologs in the deep-sea chemolithoautotroph Thiomicrospira crunogena. Appl Environ Microbiol 76: 3561–3567.

27. Dobrinski KP, Longo DL, Scott KM (2003) The carbon-concentrating mechanism of the hydrothermal vent chemolithoautotroph Thiomicrospira crunogena. J Bacteriol 185: 5761–5766.

28. Nishihara H, Igarashi Y, Kodama T (1991) Hydrogenotrophic marine genera, sp., nov., a Marine Obligately Chemolithoautotrophic Hydrogen-Oxidizing Bacterium. Int J Syst Bacteriol 41: 130–133. doi:10.1099/00221207-41-1-130.

29. Campbell BJ, Engel AS, Porter ML, Takai K (2006) The versatile e-proteobacter strain Thermodesulfovibrio kobeensis: key players in sulfidic habitats. Nat Rev Micro 4: 456–460. doi:10.1038/nrmicro1414.

30. Bradley AS, Hayes JM, Summons RE (2009) Extraordinary C-13 enrichment of diether lipids at the Lost City Hydrothermal Field indicates a carbon-limited
ecosystem. Geochim Cosmochim Acta 73: 102–118. doi:10.1016/j.gca.2008.10.005.

31. Konstantinidis KT, DeLong EF (2008) Genomic patterns of recombination, clonal divergence and environment in marine microbial populations. ISME J 2: 1052–1065.

32. Schmidt HA, Strimmer K, Vingron M, von Haeseler A (2002) TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18: 502–504.

33. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792–1797. doi:10.1093/nar/gkh340.