Diversity and Limits of Microbial Life in Submarine Hydrothermal Vent Environments - Astrobiology Implications

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Hydrothermal Vents: Parallel Habitats on Earth and Other Planets and Moons

Astrobiology focus

Follow the Water

Look for evidence of habitats that provide nutrients and energy sources (light, chemical other?)

Questions

Do the “limits” of Earth life reflect the range of habitat conditions for life elsewhere?

Can hydrothermal systems support life in the absence of photosynthesis?

Can an understanding of microbial biochemistry and physiology help in our search for life elsewhere?
Topics

• Possible parallel habitats
• Limits of life
• Magma-hosted hydrothermal environments
  – Chemistry and microbiology background
  – Black smoker microbial habitats
    • Temperature limits of life and novel microbes
  – Subseafloor environments
    • Novel primary producers and life without photosynthesis?
• Peridotite-hosted hydrothermal environments
  – Life at high pH and high temperatures
• Physiological adaptation to high temperature and subseafloor environments - the role of biofilms
• Implications
Parallel Habitats on Earth, Planets and Moons

- Subsurface - Mars and Europa
- Dry environments - Mars
- Salty ice - Europa
- Oligotrophic deep oceans - Europa
- Hydrothermal systems
  1. Magma-hosted (Mars, Europa?)
  2. Peridotite-hosted (Mars?)

Are there environments on Earth that resemble our perception of possible habitat conditions on Titan?
Implications from recent geochemical and mineralogical discoveries on Mars

- **Sources of methane**
  1. Degassing of primordial CH$_4$
  2. Degassing of fresh magma
  3. **Abiotic chemistry in hydrothermal systems** *(does the mineralogical data support hydrothermal activity?)*
  4. Life
  5. Recent cometary input
## Some Extreme Conditions for Earth Life

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extreme value in vent systems</th>
<th>Extreme value tolerated by life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magma-hosted</td>
<td>&gt;400°C in vent fluids</td>
<td>~ -20°C sea ice</td>
</tr>
<tr>
<td>Peridotite-hosted</td>
<td>&lt;20 to ~150°C</td>
<td>121°C vent archaea</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~ 60°C eukaryotes</td>
</tr>
<tr>
<td><strong>Pressure</strong></td>
<td>~ 1200 bar</td>
<td>1110 bar (trenches)</td>
</tr>
<tr>
<td></td>
<td>Deep subseafloor: ?</td>
<td>microbes and animals</td>
</tr>
<tr>
<td><strong>pH: Magma-hosted</strong></td>
<td>Low ~ 3 to ~ 7.5 (SW)</td>
<td>microbes at pH 0 to 13</td>
</tr>
<tr>
<td>Peridotite-hosted</td>
<td>pH: 9 to 12</td>
<td></td>
</tr>
<tr>
<td><strong>Toxic heavy metals</strong></td>
<td>Cu&gt;15 mM; Cd, Pb, Co ~ 1 µM</td>
<td>Cd 2-5 mM; Co 20 mM; Zn 12 mM</td>
</tr>
<tr>
<td><strong>Damaging radiation</strong></td>
<td>Alpha emissions from radium (~2000 dpm/g of barite-rich ppts)</td>
<td>5000 Gray gamma irradiation (<em>Deinococcus spp</em>)</td>
</tr>
<tr>
<td><strong>Low water activity</strong></td>
<td>50 wt% NaCl brine from phase separation</td>
<td>0.75 = 35 wt% NaCl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Haloarchaea; bacteria)</td>
</tr>
</tbody>
</table>
Limits of life issues

• Possible planetary life issues based on properties of Mars, Europa and other Jovian moons and Titan

  1. Extreme conditions not found on Earth (e.g. extreme hydrostatic pressures in the deep ocean of Europa; organic and inorganic solvents and solvent/water combinations - hypothesized for Titan)

  2. Novel biochemical structures not seen in terran life “Are the limits of Earth life the limits of all carbon-based life?”

  3. Novel metabolisms and energy sources
Magma-hosted hydrothermal environments

– Chemistry and microbiology background
– Black smoker microbial habitats
  • Temperature limits of life and novel microbes
– Subseafloor environments
  • Novel primary producers and life without photosynthesis?
Ocean Ridges, Volcanoes, and Active Hot Springs
Environmental Settings for the Sub-seafloor Microbial Biosphere
CARBON AND ENERGY SOURCES IN HYDROTHERMAL VENT ENVIRONMENTS

- **CARBON**: CO$_2$, CH$_4$, CO, organic compounds
- **NITROGEN**: Mostly as N$_2$; low levels of NH$_3$, organic-N; NO$_3^-$ reduced <20°C
- **PHOSPHORUS**: Apatite (P$_2$O$_5$) in crust; detrital P?
- **E-ACCEPTORS**: Fe(III), CO$_2$, S°, SO$_4^{2-}$, organic compounds (no O$_2$ or NO$_3^-$ >20)
- **E-DONORS**: H$_2$, H$_2$S (other reduced S compsds), CH$_4$, Reduced metals, organic compounds
Sources of Organic Material into the Subseafloor

• **In Situ Sources**
  1. Abiotic reactions: Magma driven hydrothermal systems; Serpentization reactions
  2. Biotic sources: metabolites, EPS, cell biomass, etc

• **Sea water sources**: DOC, POC, cells
Important Metabolic Groups of Microorganisms at Vents

• **Aerobic Bacteria**
  - $S$, Fe (II), $H_2$, $CH_4$ oxidizers; heterotrophs

• **Anaerobic Bacteria and Archaea**
  - Heterotrophs (all temperature ranges for growth)
  - Methanogens ($CO_2 + 3H_2 \rightarrow CH_4 + 2H_2O$)
  - Hydrogen oxidizers (coupled with S reduction and $CO_2$ fixation; CO reduction; novel metabolic pathways; bacteria and archaea)
  - Anaerobic $CH_4$ oxidation (coupled with S reduction)
  - Fe (III) reducers (coupled with oxidation of low MW organic compounds such as formate and acetate)
  - Anaerobic $NH_4^-$ oxidation with $NO_3^-$ as electron acceptor
VENT HABITATS

• Endosymbiotic to animals
• Bacterial mats (<30°C): rocks, animal surfaces, etc.
• Sediments at active vent sites
• **Active sulfide and carbonate structures**
• Chronic plumes (Event plumes)
• **Subseafloor biosphere (basalt and deep sediments)**
Chemosynthetic Symbionts

Sulfide Chimneys

Black smoker in "Strawberry Fields" Smoke & Mounds hydrothermal venting area of the Main Endeavour vent field, Endeavour segment, Juan de Fuca Ridge

Repos/dsc: 359
August 22, 1996
EXAMPLES OF MICROBIAL BIOFILMS AT VENTS

Sampling microbial mat at Axial Seamount

Microbial biofilm on mussel from 20°N EPR

Sampling Blue Mat at Axial Seamount
Filamentous Sulfur Bacteria at Vents

Scale worm (polychaete)

Marker colonized by *Beggiatoa* spp

*Beggiatoa* species

*Thiothrix* species
Representative aerobic bacteria isolated from different vent samples

Fe (II) oxidizing bacteria from diffuse flow vents

Heterotrophic bacteria from vent Alvinella polychaete gut

Chitin digesters from Riftia tube
Magma-Hosted Hydrothermal Systems

Microbial habitats:

- Diffuse flow vents - windows into the subseafloor
- Smoker fluids and sulfides
- Plumes
- Microbial mats
- Animals
- Flanks- subseafloor
Black smoker microbial habitats

• High microbial diversity and novel microbes
• Biofilms
• Temperature limits for life
Mothra Hydrothermal Field, Juan de Fuca Ridge

Faulty Towers
West Face

Mothra Hydrothermal Field,
Endeavour Segment,
Juan de Fuca Ridge

View direction: 103° T

Digital images were collected using an Imetrix 9000 electronic still camera mounted on Jason. This mosaic contains 139 images collected during 11 vertical traverses at a rate of 3 m/sec. The sulfide pinnacles host diverse macrofaunal and microbial communities supported by diffusely venting fluids that engulf many of the structures. Three sites of vigorous venting emit fluids at temperatures of 305°C.
Recovering an intact, active sulfide structure (FINN) from the Juan de Fuca Ridge
Cross Section Through a Cut-Face of Finn Showing Mineralogical Composition and Fluid Flow Patterns

**Diagram A:**
- **Z1, Z2, Z3, Z4**
- **inner conduit** — **interior** — **outer wall**

**Diagram B:**
- **302°C**
- **2°C**
- **HF**
- **SW**
- **inner flow channel**
- **clay-anhydrite+sulfide**
- **oxidized outer wall hosting tube worms**
- **chalcopyrite**
- **anhydrite**
- **pyrite, sphalerite, wurtzite (anhydrite, SiO₂)**
- **hydrothermal fluid (HF)**
- **seawater (SW)**
Microbial Biomass vs. Mineralogy within a Black Smoker Sulfide Chimney

- Zone 4
- Zone 3
- Zone 2
- Zone 1

**log cells/g (w.w.)**

- **DAPI**
- **PLFA**

Cryo-SEM

- pyr/Si zone

hydrothermal fluid

300°C

2°C seawater

10 cm

F-G2Fe1 DAPI

10 μm
Universal Phylogenetic Tree

Eukarya

Bacteria

Archaea

Euryarchaeota

(adapted from Woese, et al., 1990.)
### DAPI and FISH-direct count analysis of microbial populations within FINN

(Cells/g dry wt sulfide X10^5)

<table>
<thead>
<tr>
<th>PROBE</th>
<th>ZONE 1</th>
<th>ZONE 2</th>
<th>ZONE 3</th>
<th>ZONE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI</td>
<td>19</td>
<td>1700</td>
<td>190</td>
<td>2</td>
</tr>
<tr>
<td>ARC915</td>
<td>6 (33%)*</td>
<td>1100 (65%)</td>
<td>120 (63%)</td>
<td>0.5 (23%)</td>
</tr>
<tr>
<td>EUB338</td>
<td>10 (53%)</td>
<td>440 (26%)</td>
<td>4 (2%)</td>
<td>ND</td>
</tr>
<tr>
<td>CREN499</td>
<td>2</td>
<td>580</td>
<td>83</td>
<td>0.3</td>
</tr>
<tr>
<td>EURY498</td>
<td>3</td>
<td>250</td>
<td>28</td>
<td>0.1</td>
</tr>
<tr>
<td>TC589</td>
<td>0.2</td>
<td>30</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>MC_{688+1109}</td>
<td>0.2</td>
<td>60</td>
<td>10</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Percentage of probed cells/DAPI-stained total population (Schrenk et al., 2003)
Faulty Towers complex in the Mothra Hydrothermal Field

Archaeal 16S r RNA phylogenetic diversity data from the Finn sulfide structure (>300°C fluids)

Schrenk et al., 2003
Novel Isolates from Active Sulfide Structures

Hyperthermophilic Fe III reducer

Nannoarchaeum equitans and Ignicoccus spp

from Kashefi and Lovley, 2003

From Huber et al., 2003
Summary of results from sulfide studies

- Microorganisms are adapted to extreme conditions found in hot sulfides and diversity is controlled by mixing by hydrothermal fluid and SE

- There are intact microorganisms with DNA and ribosomes associated with mineral assemblages formed at >150°C (survival or growth?); these organisms are Archaea (mostly Crenarchaeota) that are not related to cultured organisms and distantly related other environmental clonal sequences

- New studies: in situ microbial colonization experiments using titanium probes inserted into sulfides over a temperature gradient of 2 to >300°C
Life in ocean crust - diffuse flow vents

- Extensive microbial biosphere
- High microbial diversity
- Physiologically adapted microbes to crustal environments
- Potentially important to ocean carbon cycle - subseafloor primary production
OMAN OPHIOLITE
Do ridge flanks support an active microbial community?

Ridge flanks account for 70-80% of the heat flux
Chemical fluxes may be significant
Ridge flanks 1-65 Ma make up 70% of ocean basins
Potentially enormous habitable volume on a global scale

Driving hypothesis: Where there is fluid circulation, there is microbial activity
Fluxes of fluid and heat from the oceanic reservoir

- Fluid reservoir in the crust contains 2% of the total volume of global SW
- Mean temp. in Cretaceous crust is 40°C
- Hydrothermal volume flux into the ocean approaches 20% riverine input (residence time for fluids in crust ~2700 yr)
- Ocean circulation T through crust is only 200,000 years

Porosity of upper oceanic crust as a function of age - porosity from gravity measurements, downhole logging of drill holes and ophiolite studies (Johnson and Pruiss, 2003)
Estimates of microbial biomass in crust

**Table 3. Results of metabolic energy calculations.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Aerobic sulfide oxidation</th>
<th>Anaerobic sulfide oxidation</th>
<th>Aerobic Fe (II) oxidation</th>
<th>Anaerobic Fe (II) oxidation</th>
<th>Methanogenesis</th>
<th>Sulfate reduction</th>
<th>Iron reduction</th>
<th>Knallgas reaction</th>
<th>Nitrate reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>$\text{HS}^+ + 2\text{O}_2\text{(aq)} = \text{SO}_4^{2-} + \text{H}^+$</td>
<td>$5\text{HS}^- + 8\text{NO}_3^- + 3\text{H}^+ = \text{SO}_4^{2-} + \text{N}_2 + 3\text{H}_2\text{O}$</td>
<td>$\text{Fe}^{2+} + 0.25 \text{O}_2\text{(aq)} + \text{H}^+ = 5\text{Fe}^{3+} + 6\text{H}_2\text{O}$</td>
<td>$0.5\text{N}_2\text{(aq)} + \text{Fe}^{2+} + 0.5\text{H}_2\text{O}$</td>
<td>$\text{HCO}_3^- + \text{H}^+ + 4\text{H}_2\text{O} = \text{CH}_4\text{(aq)} + 4\text{H}_2\text{O}$</td>
<td>$\text{SO}_4^- + \text{H}^+ + 4\text{H}_2\text{O} = \text{HS}^- + 4\text{H}_2\text{O}$</td>
<td>$\text{Fe}^{3+} + 0.5\text{H}_2\text{O}$</td>
<td>$\text{H}_2\text{(aq)} + 0.5\text{O}_2\text{(aq)} + \text{H}^+ = \text{NO}_3^- + 4\text{H}_2\text{O}$</td>
<td>$+ 3\text{H}_2\text{O}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Limiting reactant</th>
<th>S (rock)</th>
<th>S (rock)</th>
<th>FeO (rock)</th>
<th>FeO (rock)</th>
<th>H$_2$ (aq)</th>
<th>H$_2$ (aq)</th>
<th>H$_2$ (aq)</th>
<th>H$_2$ (aq)</th>
<th>H$_2$ (aq)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11 ± 7</td>
<td>11 ± 7</td>
<td>90 ± 60</td>
<td>90 ± 60</td>
<td>45 ± 30</td>
<td>45 ± 30</td>
<td>45 ± 30</td>
<td>45 ± 30</td>
<td>45 ± 30</td>
</tr>
<tr>
<td>Stoichiometric factor</td>
<td>0.714</td>
<td>0.286</td>
<td>0.8</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corr. S, Fe (10$^{10}$ mol/yr)</td>
<td>7.9 ± 5.0</td>
<td>3.2 ± 2.0</td>
<td>68 ± 43</td>
<td>23 ± 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔG° (kJ)</td>
<td>−795</td>
<td>−3785</td>
<td>−51.3</td>
<td>−232</td>
<td>−231</td>
<td>−262</td>
<td>−80.8</td>
<td>−264</td>
<td>−752</td>
</tr>
<tr>
<td>ΔG (kJ/mol S, Fe, H$_2$)</td>
<td>−751</td>
<td>−87.4</td>
<td>−66.2</td>
<td>−287</td>
<td>−21.6</td>
<td>−67.6</td>
<td>−36.1</td>
<td>−199</td>
<td>−134.4</td>
</tr>
<tr>
<td>Energy (10$^{12}$ kJ/yr)</td>
<td>59 ± 38</td>
<td>25 ± 17</td>
<td>48 ± 30</td>
<td>10 ± 7</td>
<td>2.4 ± 1.6</td>
<td>7.5 ± 5.1</td>
<td>33 ± 22</td>
<td>90 ± 61</td>
<td>60 ± 40</td>
</tr>
<tr>
<td>kJ/g C cellular mass$^d$</td>
<td>292 ± 117</td>
<td>292 ± 117</td>
<td>292 ± 117</td>
<td>292 ± 117</td>
<td>83 ± 33</td>
<td>83 ± 33</td>
<td>83 ± 33</td>
<td>83 ± 33</td>
<td>83 ± 33</td>
</tr>
<tr>
<td>Biomass (10$^{10}$ g C/yr)</td>
<td>20 ± 15</td>
<td>88 ± 6.5</td>
<td>16 ± 11</td>
<td>3.3 ± 2.8</td>
<td>2.9 ± 2.2</td>
<td>9.2 ± 7.1</td>
<td>39 ± 30</td>
<td>108 ± 84</td>
<td>73 ± 56</td>
</tr>
<tr>
<td>Biomass (10$^{10}$ g dry wt/yr)</td>
<td>44 ± 33</td>
<td>19 ± 14</td>
<td>36 ± 26</td>
<td>7.8 ± 6.1</td>
<td>6.4 ± 4.8</td>
<td>20 ± 16</td>
<td>86 ± 68</td>
<td>237 ± 184</td>
<td>160 ± 123</td>
</tr>
<tr>
<td>% anaerobic heterotrophic production$^e$</td>
<td>15 ± 10%</td>
<td>5.5 ± 4.0%</td>
<td>10 ± 8%</td>
<td>2.2 ± 1.7%</td>
<td>1.8 ± 1.4%</td>
<td>5.7 ± 4.8%</td>
<td>24 ± 19%</td>
<td>67 ± 52%</td>
<td>45 ± 35%</td>
</tr>
</tbody>
</table>

From Bach and Edwards, 2003

Potential microbial biomass production within ridge flanks up to $\sim 1 \times 10^{12}$ g C/yr
(Phytoplankton PP is $\sim 43 \times 10^{15}$ g C/yr)
Sampling the Subseafloor Crust for Microorganisms

- Drilling
- New seafloor eruptions (ejection of organisms from crust)
- Diffuse-flow vents
- Sulfide chimneys ("black smokers")
- Metal darts
- possibly new technology involving rocket-powered penetrators
CoAxial Eruption

- thermophiles cultured

Floc site
(deep heat source)
18°C

Flow site
(shallow heat source)
36°C

Magma Supply

modified from Holden et al., 1998
Microscopic Observations of “Subseafloor” Organisms Following a Deep-Sea Volcanic Eruption

Evidence from EM:
Iron oxidation
Methane oxidation
Sulfur oxidation
Protists

Organisms cultured:
(Sub-seafloor Indicators)
Methanogens (55-90°C)
Iron reducers (55-90°C)
Heterotrophs (55-90°C)

Ultra thin-section TEM’s of organisms from fluids collected from CoAxial after a new eruption (a), 0.5 µm, (b), 1µm (from Holden and Adams, 2003)
North Gorda Eruption

- thermophiles cultured
GR1 cultured with FeOOH showing attachment (F) and biofilm formation (C, D); TEM showing flagella (E)
Subseafloor Indicator Organisms (crustal weeds)

• Anaerobic thermophiles and hyperthermophiles from cold to warm oxygenated waters (min. growth T approximately 50°C)

• Heterotrophs (many obligate S° reducers) and methanogens (numbers generally less than 1% of total counts)
Subseafloor at Marker 33

Seawater Dominated
- Aerobic; 2-10 °C
- Abundant seawater electron acceptors, $\text{NO}_3^-$, $\text{O}_2$, $\text{SO}_4^{2-}$; $\gamma$, $\beta$, and $\varepsilon$-proteobacteria, possibly seawater organisms

Heated Crustal Fluid
- Seawater and hydrothermal
- Aerobic/Anaerobic; 10-50 °C
- $\text{SO}_4^{2-}$, $\text{CO}_2$ reduction;
- Fe, $\text{H}_2$, $\text{CH}_4$, $\text{H}_2\text{S}$, $\text{CH}_4$ oxidation;
- Methanococcales, $\varepsilon$-proteobacteria,
- Candidate Divisions ABY1 and WS6

Hydrothermal Dominated
- Anaerobic; 50-110 °C
- $\text{CO}_2$, $\text{CH}_4$; Sulfur, and Fe(III) reduction;
- $\text{H}_2$ and $\text{CH}_2\text{O}$ electron donors;
- Thermococcales, Methanococcales,
- Desulfurobacterium, and
- Thermodesulfobacterium

Huber et al. 2003
Canonical characteristics of subseafloor microbial communities—subseafloor indicator organisms

- **Community diversity:** different from SW communities
- **Physiological adaptation of isolates**
  - Exploit nutrients from rocks; tolerate metals
  - Form biofilms on mineral surfaces
  - Use Fe (III) and S as electron acceptors
  - Heterotrophs are oligotrophic
  - Fix CO$_2$ and oxidize hydrogen
  - Wide temperature growth range
  - High temperature organisms are anaerobic
The Subseafloor Biosphere-An active microbial ecosystem that it independent of photosynthesis?

• What phylogenetic groups of microorganisms are the “primary producers” and what metabolic pathways do they use? (CO₂ fixation, heterotrophy, etc)

• What are the sources and kinds of electron acceptors used by subseafloor microorganisms?

• What are sources of N, P and organic matter used by subsurface microorganisms?
Ax99-59 isolated from Axial Volcano

- Strict anaerobe
- Thermophilic
- CO$_2$ is carbon source
- H$_2$ as energy source
- Reduces sulfur species
- 32 min doubling time under optimal conditions
- G+C ratio if 40%
- New genus in the Aquifacales

*Also ε-Proteobacteria may be important primary producers

Scanning electron micrograph of Ax99-59. Under most culturing conditions this organism produce copious amount of exo-polysaccharide, which may be involved in Biofilm formation. Scale bar is 1 µm
Some characteristics of the \(\varepsilon\)-Proteobacteria

- Ubiquitous in vent environments
- Anaerobic; microaerophilic; some use \(O_2\) and \(NO_3^-\) as e-acceptors
- Mesophilic and thermophilic
- Reduce S compounds
- Metabolism similar to Aquificales (e.g. reductive TCA cycle)

Figure from Longnecker and Reysenbach, 2001
Scale bar is 20µm
Conclusions

• There exists an indigenous subseafloor community of bacteria and archaea that are indicative of both an anaerobic “hot” subseafloor habitat and an intermediate “warm” subseafloor habitat

• There is potentially very high microbial productivity in the subseafloor near active spreading areas

• The bacterial and archaeal diversity at Marker 33 is higher in the particle-attached fraction and increases over time; this is particularly true for the ε-proteobacteria

• The subseafloor harbors microorganisms that have novel metabolism specifically adapted to the subseafloor
End of presentation 1
Part 2

- Novel primary producers in vent environments - Life without photosynthesis?
- Lost City peridotite hosted hydrothermal environment - a source of methane to Mars?
- Biofilms and possible life in super-heated environments
- Astrobiology comments
Ancient Metabolic Pathways and the “Unity of Metabolism”

- Reductive TCA Pathway - reduce CO$_2$ - 
  *dominant in magma-hosted vent environments*

- Reductive AcetylCoA Pathway - reduce CO$_2$ to CH$_4$ and in reverse oxidize CH$_4$ to (CH$_2$O)$_n$ - 
  *Lost city primary producer*

- Oxidation of C$_1$ and C$_2$ compounds with Fe(III) or S° - 
  *dominant in magma-hosted vent environments*

- At least two unknown CO$_2$-fixing pathways in Archaea - *all from vent environments*
The four known CO$_2$ fixation pathways

A. Calvin Benson Pathway (oxygenic photosynthesis; most chemolithoautotrophic bacteria)

B. Reductive TCA Pathway (important pathway in vent organisms including novel archaea and epsilon-proteobacteria)

C. Reductive Acetyl-CoA Pathway (methanogens)

D. 3-hydroxypropionate cycle, *Chloroflexus* species
Autotrophic $\text{CO}_2$ fixation pathways in Archaea (Crenarchaeota) From Hügler et al., 2003

[Diagram showing phylogenetic relationships and different pathways for CO2 fixation in Crenarchaeota]
Ax99-59 isolated from Axial Volcano

- Strict anaerobe
- Thermophilic
- CO\textsubscript{2} is carbon source
- H\textsubscript{2} as energy source
- Reduces sulfur species
- 32 min doubling time under optimal conditions
- G+C ratio if 40%
- New genus in the Aquifacales

Huber, unpublished

Scanning electron micrograph of Ax99-59. Under most culturing conditions this organism produce copious amount of exo-polysaccharide, which may be involved in Biofilm formation. Scale bar is 1 \textmu m
How is Ax99-59 fixing CO₂?

- 4 known paths of CO₂ fixation in prokaryotes—certainly other novel pathways, i.e. *Igniococcus* spp.
- Reductive Citric Acid Cycle (rTCA)—potentially most ancient autotrophic CO₂ fixation pathway
- rTCA is important at vents:
  - Genes detected in vent isolates *Aquifex* spp, ε-proteobacteria and hyperthermophilic Crenarchaeota
  - Genes detected and expressed in vent environmental samples

Wächtershäuser, 1990; Beh *et al.* 1993; Cody *et al.* 2001; Hügler *et al.* 2003; Campbell *et al.* 2003, 2004
Can active life exist in the absence of photosynthesis

- Novel anaerobic primary producers use hydrothermally derived carbon sources ($\text{CO}_2$, CO, $\text{CH}_4$, organic compounds synthesized abiotically) and novel metabolic pathways
- Hydrogen is the most important energy source
- $\text{S}^\circ$, Fe(III), and $\text{CO}_2$ most important e-acceptors (need to know more about the S and iron cycles in vent environments)
- High diversity of $\text{N}_2$-fixing bacteria and archaea
- Need to understand more about the P cycle in vents
Topics

• Lost City Hydrothermal Field - location and characteristics
• Carbon and energy sources for microbes at Lost City - serpentinization reactions
• Unique challenges for life at Lost City
• Microbial diversity in Lost City carbonates and fluids
• The unclear picture of the microbial ecology at Lost City
Lost City Hydrothermal Field

- Ultramafic oceanic crust
- Fluid flow sustained by sub-seafloor *serpentinization* reactions
- High pH, moderate temperature, volatile-rich fluids
- Tall carbonate chimneys (60 m) and estimated to be >30,000 years old
SOURCE REACTIONS FOR HYDROGEN

$\text{(MgFe)}_2\text{SiO}_4 + \text{H}_2\text{O} \rightarrow \text{olivine}$

$\text{Mg}_3\text{Si}_2\text{O}_5 (\text{OH})_4 + \text{Fe}_3\text{O}_4 + \text{H}_2$

$\text{serpentine} \quad \text{magnetite}$

$\text{Fe}_2\text{SiO}_4$ (fayalite) is the active $\text{H}_2$ producing compound of olivine

Hydrogen in combination with Ni and Fe-bearing minerals can result in the synthesis of methane and other low MW C-compounds.
Rock-hosted Hydrothermal Environments at the LCHF

Hydrothermal fluids

- T (°C): 40 - 90
- pH: 9-11
- H₂, CH₄
- low metals, H₂S
- gradient environment

- T (°C): 90 - 150?
- pH: 11+
- H₂, CH₄, organics?
- highly reducing

carbonate chimneys

Peridotite

'serpentinization front'
### Key bio-energetic parameters of fluids associated with serpentinization

<table>
<thead>
<tr>
<th>Constituent/property</th>
<th>Experimental/theoretical</th>
<th>Lost City</th>
<th>Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature °C</td>
<td>25 - 300</td>
<td>40 - 93</td>
<td>7</td>
</tr>
<tr>
<td>pH</td>
<td>8 - 12</td>
<td>9 - 11</td>
<td>8</td>
</tr>
<tr>
<td>H₂ (mmol kg⁻¹)</td>
<td>1 - 100</td>
<td>0.25-0.43</td>
<td>0</td>
</tr>
<tr>
<td>CH₄ (mmol kg⁻¹)</td>
<td>0.01 - 1</td>
<td>0.13-0.28</td>
<td>0</td>
</tr>
<tr>
<td>CH₄/C₂H₂+C₃H₆</td>
<td>10³ - 10⁴</td>
<td>100:1</td>
<td>-</td>
</tr>
<tr>
<td>H₂S (mmol kg⁻¹)</td>
<td>0.1 - 1</td>
<td>0.064</td>
<td>0</td>
</tr>
<tr>
<td>SO₄²⁻ (mmol kg⁻¹)</td>
<td>0</td>
<td>5.9-12.9</td>
<td>28.6</td>
</tr>
<tr>
<td>CO₂ (mmol kg⁻¹)</td>
<td>0</td>
<td>not detected</td>
<td>2.30</td>
</tr>
<tr>
<td>Total Fe (µmol kg⁻¹)</td>
<td>1.0</td>
<td>Not detected</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### End-member Hydrothermal Environments

#### Sulfide systems
- Driven by the cooling of magma/rock
- High T (up to 400°C)
- Low pH fluids
- High metal concentrations
- Variable volatile concentrations
- Metal-sulfide chimneys

#### Carbonate systems
- Driven by exothermic water:rock rxns
- Moderate T (<90°C)
- High pH fluids
- Low metal concentrations
- High volatile concentrations
- Carbonate chimneys
## Some Extreme Conditions for Life

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extreme value in vent systems</th>
<th>Extreme value tolerated by life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magma-hosted: Lost City</td>
<td>&gt;400°C in vent fluids</td>
<td>~ -20°C sea ice</td>
</tr>
<tr>
<td></td>
<td>&lt;20 to ~150°C</td>
<td>121°C vent archaea</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~ 60°C eukaryotes</td>
</tr>
<tr>
<td><strong>Pressure</strong></td>
<td>~ 1200 bar</td>
<td>1110 bar (trenches)</td>
</tr>
<tr>
<td></td>
<td>Deep subseafloor: ?</td>
<td>microbes and animals</td>
</tr>
<tr>
<td><strong>pH: Magma-hosted</strong></td>
<td>Low ~ 3 to ~ 7.5 (SW)</td>
<td>microbes at pH 0 to 13</td>
</tr>
<tr>
<td>Lost City</td>
<td>pH: 9 to 12</td>
<td></td>
</tr>
<tr>
<td><strong>Toxic heavy metals</strong></td>
<td>Cu&gt;15 mM; Cd, Pb, Co ~ 1 μM</td>
<td>Cd 2-5 mM; Co 20 mM; Zn 12 mM</td>
</tr>
<tr>
<td><strong>Damaging radiation</strong></td>
<td>Alpha emissions from radium (~2000 dpm/g of barite-rich ppts)</td>
<td>5000 Gray gamma irradiation (<em>Deinococcus</em> spp)</td>
</tr>
<tr>
<td><strong>Low water activity</strong></td>
<td>50 wt% NaCl brine from phase separation</td>
<td>0.75 = 35 wt% NaCl (Haloarchaea; bacteria)</td>
</tr>
</tbody>
</table>
Key Microbiological Questions at the LCHF

• Can serpentinization support microbial ecosystems?  
  - What are the pathways of carbon fixation?  
  - What are the energy sources?

• What is the microbial diversity associated with novel, extreme ecosystems which are; highly-reducing, high pH, and at hyperthermophilic temperatures?

• What are the physiological/metabolic/biochemical properties of organisms sustained by hydrothermal activity at the LCHF?
Methods

• Epifluorescence microscopy: DNA stains; Fluorescent in situ hybridization (FISH)
• Scanning electron microscopy
• Microbial diversity based on 16S rRNA analyses
• Identification and expression of specific genes using molecular methods
• Culture and characterization of specific groups of microorganisms
• Related work by others: fatty acid and stable isotope analyses
Microscopic observations of carbonate structures

- Biofilms dominate

- Different morphotypes of microorganisms found on outer and inner sections of the carbonate structures

- Methanosarcinales related organisms produce $\text{F}_{420}$ (associated with methane metabolizers)

- Microbial abundances ($10^5$ to $\sim10^8$/gm carbonate)
Some predictions about the metabolic diversity of microorganisms at Lost City

- If CO$_2$ is available, methane producers could be significant (CO$_2$ + H$_2$ or use acetate or methylamines)
- Aerobic and anaerobic CH$_4$ and H$_2$ oxidizers should exist
- Sulfate reducers should exist (SW SO$_4^{2-}$ + H$_2$ with acetate or other organic acids if CO$_2$ is limiting)
- If sulfate reduction occurs could have sulfide oxidation with O$_2$ or NO$_3^-$ if CO$_2$ is available (SW e-acceptors)
Low archaeal diversity in active carbonate chimney samples from Lost City.

a. $F_{420}$ autofluorescence of a biofilm
b. Biofilm cells encased in EPS
c. $F_{420}$ autofluorescence of methane-metabolizing Archaea
d. FISH photomicrograph confirming that the $F_{420}$-fluorescent cells are Archaea

16S rRNA tree from Schrenk and Brazelton, unpublished.
Detection of mcrA genes in Lost City carbonate samples indicate the presence of a distinct \textit{Methanosarcinales} phylotype and a group related to AMME-1.

\begin{center}
\begin{tikzpicture}
\node (root) {\textbf{Methanogenic pathway}};
\node (coenzyme) [below of=root] {\textbf{Coenzyme}};
\node (f420) [below of=coenzyme] {\textbf{$F_{420}$}};
\node (steps) [below of=f420] {\textbf{Many steps}};
\node (ch4) [below of=steps] {\textbf{CH$_4$-S-CoM}};
\node (methyl-coenzyme) [below of=ch4] {\textbf{Methyl-coenzyme \textit{M} reductase}};
\node (ch) [below of=methyl-coenzyme] {\textbf{CH}};
\node (4) [below of=ch] {\textbf{4}};

\draw [->] (root) -- (coenzyme) node [midway, above] {$\text{CO}_2$};
\end{tikzpicture}
\end{center}
Microbe-microbe interactions and solving the decades old problem of anaerobic methane oxidation in marine environments

Methanogenic pathway

\[ \text{CO}_2 \downarrow \]

Many steps

\[ \text{Coenzyme F}_{420} \]

\[ \text{CH}_4 \rightarrow \text{S-CoM} \]

Methyl-coenzyme M reductase

\[ \text{CH}_4 \]

4

Methanosarcinales are metabolically versatile - How do they make a living in situ?

- They make methane from CO$_2$ and H$_2$, or from acetate, methylamines, etc.
- Some groups can oxidize methane anaerobically usually in conjunction with sulfate reducing bacteria.
- Described species of *Methanosarcina* have maximum growth T’s of 55°C (Lost City group found in 90°C samples).
- Genes involved in methanogenesis (*mcrA*) and nitrogen-fixation (*nifH*) have been detected in Lost City *Methanosarcina*-related organisms from in situ samples and cultures.
Archaeal phylogenetic groups detected in carbonate chimneys from Lost City

<table>
<thead>
<tr>
<th>Phylogenetic affiliation</th>
<th>Clone</th>
<th>$n_{\text{ribo}}^a$</th>
<th>Closest 16S rDNA match$^b$ (accession no.)</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Euryarchaeota</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanosarcinales</td>
<td>LC1022-al</td>
<td>9</td>
<td>Gas hydrate clone</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>LC1149-a56</td>
<td>18</td>
<td>Gas hydrate clone</td>
<td>93</td>
</tr>
<tr>
<td><strong>Crenarchaeota</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Marine Group 1)</td>
<td>LC1231-a51</td>
<td>1</td>
<td>Pacific Ocean clone</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>LC1231-a68</td>
<td>7</td>
<td>Pacific Ocean clone</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>LC1231-a76</td>
<td>1</td>
<td>Pacific Ocean clone</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>LC1231-a78</td>
<td>5</td>
<td>Deep-sea sediment</td>
<td>98</td>
</tr>
</tbody>
</table>

$a_{\text{ribo}}$ indicates the no. if distinct patterns per sequence type determined by RFLP
## Microbiological characteristics of hydrothermal carbonate samples from Lost City

<table>
<thead>
<tr>
<th>Active venting structures</th>
<th>$T^\circ C$</th>
<th>Porosity (%)</th>
<th>Cell counts (cells/g)</th>
<th>Proportion (FISH, %)</th>
<th>Archaea</th>
<th>Eubacteria</th>
<th>LCMS$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimney</td>
<td>&gt;7</td>
<td>40 - 50</td>
<td>5.6(0.9)$\times 10^6$</td>
<td>7.5</td>
<td>14.8</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Chimney (in)</td>
<td>75</td>
<td>&gt;40</td>
<td>2.0(0.4)$\times 10^6$</td>
<td>31.4</td>
<td>3.5</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>Chimney (out)</td>
<td>&lt;75</td>
<td>&lt;40</td>
<td>8.6(0.2)$\times 10^7$</td>
<td>11.2</td>
<td>26.4</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Flange (in)</td>
<td>55</td>
<td>40 - 50</td>
<td>3.1(0.3)$\times 10^8$</td>
<td>37.2</td>
<td>4.2</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Flange (out)</td>
<td>&lt;55</td>
<td>30 - 40</td>
<td>2.7(0.4)$\times 10^8$</td>
<td>5.7</td>
<td>23.1</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Extinct structures (4)</td>
<td>7</td>
<td>15 - 30</td>
<td>$10^6 - 10^7$</td>
<td>3 - 7</td>
<td>10 - 20</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

$^a$LCMS are cells that hybridized with probe LCMS860 targeting the phylotype found at Lost City.
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Microbiology - Summary of results

- Methanosarcinales dominate the archaeal libraries - their metabolism is unclear
- Sulfate reducers (delta-proteobacteria) generally not detected
- mcrA amino acid sequences from active carbonate structures indicate methanogenesis
- Stable carbon isotopic analyses of archaeal lipids ambiguous because of low availability of CO$_2$
- Too early to determine metabolism from culture data but indicate versatile physiology (T, pH etc)
**Microbial diversity in Lost City carbonates and magma-hosted sulfide chimneys: a comparison**

### Lost City carbonates
- Microbial community diversity varies with location in carbonates
- *Microbial communities at pH >10 and Temperatures 80-90°C*
- One archaeal phylotype dominates
- Low bacterial diversity dominated by groups seen in magma-hosted vent systems
- Biofilms dominate

### Sulfide chimneys
- Microbial community diversity varies with location in sulfide
- *Evidence of intact microbes at temperatures >150°C*
- Extremely high diversity of “uncultured” archaea
- Bacteria dominate on outer wall and archaea dominate inner sections of sulfides
- Biofilms dominate
Summary and conclusions

- Crustal microbial communities are phylogenetically and physiologically diverse and include all thermal groups.
- The primary producers utilize $\text{H}_2$ as the key energy source.
- Sulfur and Fe(III) are important e-acceptors.
- The subseafloor may be a source or microbes to the deep sea.
Biofilms at Vents

- Microscopic and macroscopic observation on rocks and sulfides
- Most thermophilic and hyperthermophilic bacteria and archaea isolated from subseafloor and sulfides form biofilms
- The “primary producers” isolated from subseafloor environments form biofilms
The attached physiology is the ‘way of life’ in endolithic deep-sea systems

Schrenk et al., unpublished

Edwards et al., GCA, 2003
Biofilms responses to environmental stress

Key Questions

• How do attached communities respond to environmental stresses?

• How do they compare physiologically to organisms grown under optimal conditions?

• What are the limits to cell viability?

• What bio-molecules are preserved under sub-optimal conditions?

• Can we use the concept of “reverse chemical evolution” to determine the bio-molecular upper temperature limits to life?
A range of physiologies evolve within the biofilm communities, even though they originate from mono-clonal cultures.

*Schrenk, et al., unpublished*
30 μm
Novel approaches to microbial ecology
Hydrothermal Vents: Parallel Habitats on Earth and Other Planets and Moons

Astrobiology focus
Follow the Water
Look for evidence of habitats that provide nutrients and energy sources (light, chemical other?)

Questions
Do the “limits” of Earth life reflect the range of habitat conditions for life elsewhere?
Can hydrothermal systems support life in the absence of photosynthesis?
Can an understanding of microbial biochemistry and physiology help in our search for life elsewhere?
Earth - some issues

1) molecular and physiological diversity of the uncultured majority of microorganisms
2) Molecular and biochemical diversity of microbes that escape detection using tests based on “universal” gene sequences
3) Parallel environments on Earth, planets and moons that have not been adequately studied
4) Earth environments too extreme for terran biochemistry (combination effects such as high T and high pH or high salt)

– Are there microorganisms that can use energy sources other than light and chemical energy?
Life related properties of Europa

- Chemistry: High concentrations of MgSO$_4$ hydrates (The need to know more about the sulfur cycle on Europa)
- CO$_2$ at approximately 0.2%
- Evidence for H$_2$O$_2$ (possible production of formate)
- Anoxic
- Temperatures: -57 to -7°C
- Pressures >10,000 atm?
Implication of subsurface life on Europa supported by hydrothermal systems

• If hydrothermal activity does occur on Europa than subseafloor life as we know it on Earth could exist (nitrogen and P sources are issues)

• Anaerobic organisms that fix CO$_2$ using H$_2$ and oxidized forms of S, and methanogens would dominate (need to know more about the sulfur cycle on Europa)

• The result would be 4 billion years of subsurface microbes being ejected into the water column where they would remain dormant and eventually reach high numbers - The ocean on Europa could be a cesspool
Some final comments

We don’t understand the origin of life on Earth and the possibility of different origins on other planets and moons.

We don’t understand the full range of possible biochemistries of carbon-based life.

We assume a “unity of metabolism” on aqueous solar bodies based on geo-properties.
The water here gushed from volcanic-tectonic fissures. While the fissures themselves may be older, the latest eruption was probably only about 10 million years ago.

"The water here gushed from volcanic-tectonic fissures. While the fissures themselves may be older, the latest eruption was probably only about 10 million years ago."

"Given the discovery of so many man-made monuments on Mars — including canals — the sighting of a boat would not in and of itself be surprising," notes Dr. van der Haas. "But it remains to be seen if there is a boat surviving from that time, as the canals were built by ancient civilizations that may not have been here until the late 20th century."

Meanwhile, church leaders say the discovery shouldn't trouble Bible believers.

"Whatever else, declared Rev. Harper, "this confirms once again that the Bible is true."