# Microbial Cultivations from Mid-Atlantic Ridge Hydrothermal Vent Plumes

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## Summary:

Hydrothermal vent plumes seed the deep ocean with reduced forms of metals such as iron, manganese, and reduced sulfur. Often, these reduced chemical species fall out into the surrounding sedimentary environment where they are oxidized by chemolithotrophic bacteria. However, neutrally buoyant vent plumes can carry these reduced elements far from their source, driven by deep oceanic currents, making them available for water column associated microbial species. Little is known about the extent of biotic vs. abiotic oxidation of reduced chemical species in plumes. Here, data is presented from a cultivation-based study of iron-cycling bacteria from three different vent plumes along the Mid-Atlantic Ridge (Figure 1). Initial microbial growth was observed in media by oxidation of a semi-solid FeS/agar plug at faster rates than negative controls and other enrichments (Figure 3a, 3b). Extraction and sequencing of 16S rRNA revealed an enriched co-culture of *Shewanella* and *Moritella* species that have been previously observed in subseafloor oceanic crust environments (Table 1, Figure 4). Their involvement with metal cycling is under investigation, as these are typically heterotrophic or iron-reducing organisms.

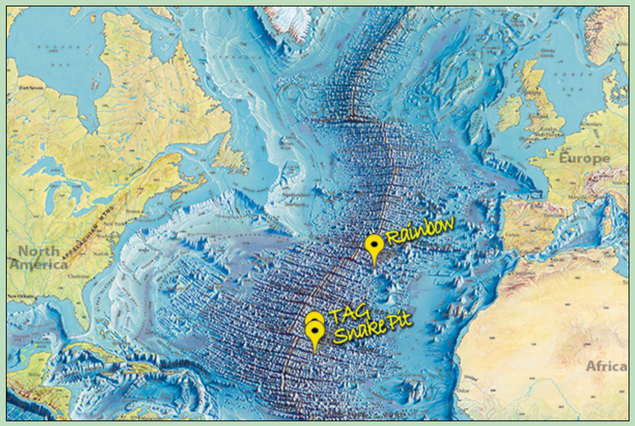


**Figure 1: Locations of three sampled vent plumes along the Mid-Atlantic Ridge.**

## Methods:

Hydrothermal plume water was collected via Niskin bottle during R/V Knorr cruise KN209-02 in October/November 2012. Five mls of plume water was used as inoculum in standard Fe-oxidizing (FeOB) media, sulfide-oxidizing media, and media containing laboratory-synthesized pyrite nanoparticles mimicking those flowing from the vent (Table 2). FeOB media contained a 1% wt/vol agarose plug prepared with a 1:1 ratio of washed FeS and 0.2 µm filtered, autoclaved seawater underlying a semi-solid 0.15% agarose layer made with filtered, autoclaved seawater. Media was stored anaerobically until use. Culture vessels (20 ml crimp-sealed serum vials) were inoculated on ship immediately after retrieval of Niskin bottles. Enrichments were kept at 10 degrees for several months and exposed to oxygen weekly via a 22-gauge needle inserted through the rubber septa for several minutes. Significant oxidation of the FeS plug compared to negative controls was observed in an enrichment from the Rainbow vent plume. DNA was extracted from this enrichment in July 2013 via the *MoBIO UltraClean Microbial DNA* kit (MoBIO Laboratories Inc., Carlsbad, CA). The 16S rRNA gene was amplified with full-length general bacteria primers. PCR products were cloned via the *TOPO TA Cloning Kit for Sequencing* (Life Technologies). Vector positive clones were sent for sequencing at Genewiz, Inc. (South Plainfield, NJ). Sequences were trimmed in *Sequencher 4.8* (Gene Codes Corporation, Ann Arbor, MI) and 17 high quality clones were used for further phylogenetic analysis.

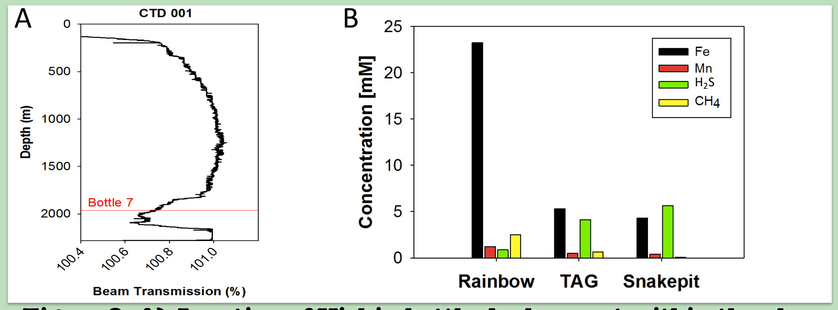


**Figure 2: A) Location of Niskin bottle deployment within the plume as indicated by transmissivity data. B) Concentration of various ions across the three sampled vent plumes.**

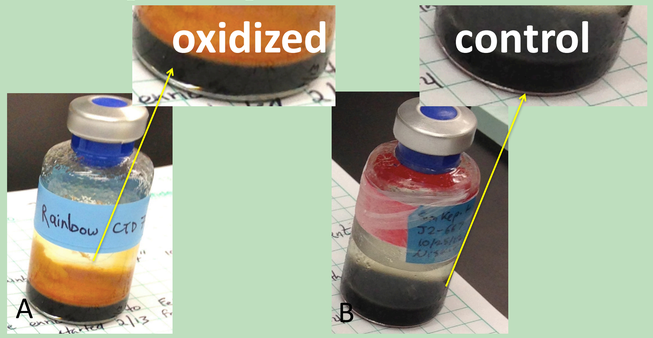


**Figure 3: A positive growth bottle indicated by a defined oxidation of the iron-sulfide agar plug (A) as compared to an uninoculated or negative growth bottle (B).**

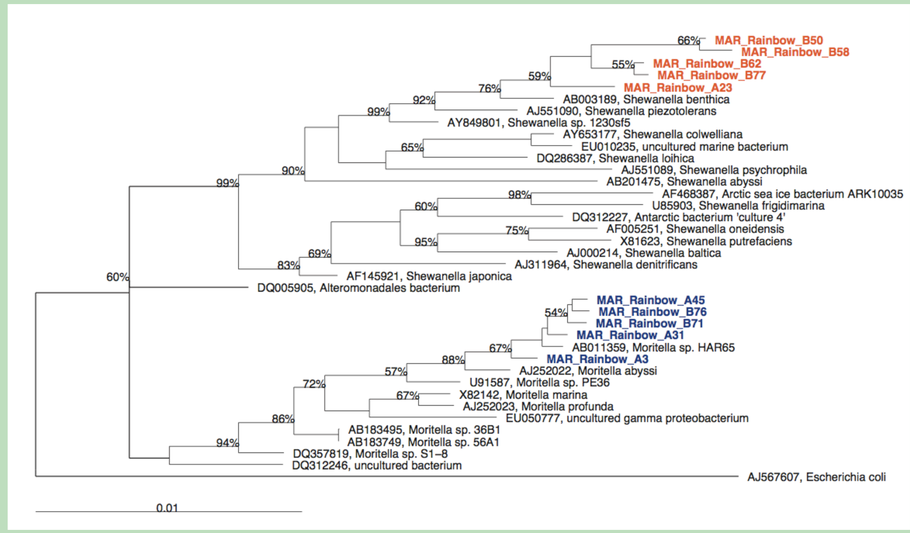


**Figure 4: Phylogenetic tree of selected clones from the *Shewanella sp.* group (red) and the *Moritella sp.* group (blue). Tree was constructed using neighbour-joining phylogeny with 1,000 bootstrap replicates. Only boostrap values above 50% are shown. Cultivated isolates show large intragroup heterogeneity and are distinct from previous cultivated lineages.**

## Results:

| | Clone | Top Hits | Environment Previously Seen |
|---|---|---|---|
| *Shewanella* sp. | A_23 | Uncultured bacterium clone FLOCS_1301A-GWPyrrhotite_D02 | FLOCS: Pyrrhotite enrichment, Juan de Fuca Ridge |
| | B_50 | Uncultured bacterium clone FLOCS_1301A-GWPyrrhotite_D02 | FLOCS: Pyrrhotite enrichment, Juan de Fuca Ridge |
| | B_56 | Uncultured bacterium clone FLOCS_1301A-GWPyrrhotite_D02 | FLOCS: Pyrrhotite enrichment, Juan de Fuca Ridge |
| | B_58 | Uncultured bacterium clone FLOCS_1301A-Basalt_C09 | FLOCS: Basalt enrichment, Juan de Fuca Ridge |
| | B_61 | Uncultured bacterium clone FLOCS_1301A-GWPyrrhotite_D02 | FLOCS: Pyrrhotite enrichment, Juan de Fuca Ridge |
| | B_62 | Uncultured bacterium clone FLOCS_1301A-Basalt_C09 | FLOCS: Basalt enrichment, Juan de Fuca Ridge |
| | B_77 | Shewanella sp. DB172R | deep-sea sediment, Izu-Bonin trench |
| *Moritella* sp. | A_27 | Uncultured bacterium clone EPR3967-O2-Bc74 | oceanic crust |
| | A_28 | Moritella sp. J28 | western North pacific ocean |
| | A_3 | Uncultured deep-sea bacterium | deep-sea surface sediments, South Atlantic |
| | A_31 | Moritella marina | bacterial mat at 3100m in Japan Sea |
| | A_34 | Moritella sp. J28 | western North pacific ocean |
| | A_45 | Uncultured gamma proteobacterium | hydrothermal vent field, Mid-Atlantic ridge |
| | A_9 | Uncultured gamma proteobacterium | hydrothermal vent field, Mid-Atlantic ridge |
| | B_54 | Uncultured bacterium clone EPR4055-N3-Bc26 | oceanic crust |
| | B_71 | Uncultured gamma proteobacterium | hydrothermal vent field, Mid-Atlantic ridge |
| | B_76 | Uncultured bacterium clone EPR4055-N3-Bc26 | oceanic crust |

**Table 1: Top BLAST hits for each clone from the nr/nt database and the previously found environments for each top hit.**

| Media | Rainbow | TAG | Snakepit |
|---|---|---|---|
| FeOB Media (FeS agar plug) | ✔ | ✖ | ✖ |
| Sulfide-oxidizing | ✖ | ✖ | ✖ |
| Laboratory synthesized pyrite nanoparticles | ✖ | ✖ | ✖ |

**Table 2: Result of each cultivation strategy at each vent site. ✔ = positive growth, ✖ = no visible growth.**

- **An enriched co-culture of two microbial species, *Shewanella sp.* and *Moritella sp.*, arose from cultivation efforts using media specific for iron oxidizers. *Shewanella sp.* are known as iron reducers.**
- **Enrichments for sulfide oxidizing microbes did not show any growth. No growth was observed in enrichments for iron oxidizing microbes using laboratory-synthesized pyrite nanoparticles as an iron source. Pyrite nanoparticles of similar structure have been observed emanating from hydrothermal vents (Yücel et al. 2011).**

## Discussion:

- **The only enrichment to show significant growth in iron oxidizer media was from the site with the highest plume water reduced iron concentration. Homologs of iron reduction proteins found in *Shewanella* have been found to work in reverse, oxidizing iron (Liu et al. 2012). Thus, in our enrichment, *Shewanella* may be oxidizing iron, reducing the oxidized iron species atop the remaining reduced iron-sulfide agar plug (Fig. 3A), or living as a heterotroph considering its co-enrichment with a *Moritella* species which are known heterotrophs. On-going investigations seek to clarify this.**

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References:

Liu, Juan et al. 2012. Identification and characterization of MtoA: a decaheme c-type cytochrome of the neutrophilic Fe(II)-oxidizing bacterium *Sideroxydans lithotrophicus* ES-1. Frontiers in Microbiology. Vol. 3 (37); 1-11

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