Investigating Bacterial Communities on the 4850 ft. Level of the Sanford Underground Research Facility

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Introduction

1470 meters underneath the surface, in tunnels of the Sanford Underground Research Facility (SURF) at Lead, SD, warm (37ºC), anaerobic fracture water from deep aquifers, flowing through minerals such as quartz, chlorite-chamosite, and annite, emerges from the rock and encounters oxygen as it forms pools of drainage water in the "17 Ledge". Although the water is low in dissolved organic matter, methane, sulfide, ferrous iron and nitrite within the pools of alkaline water allow both chemoautotrophic and chemoheterotrophic microbes to thrive [1][2][3].

Bollman's technique of diffusion chambers[4] (media surrounded by permeable membranes) allows for the isolation of prokaryotic organisms that otherwise could not readily be cultured by conventional means.

In this experiment, we use a combination of 16S rDNA sequencing, direct culturing of bacteria on low-nutrient media, and culturing using diffusion chambers to investigate bacteria in a pool of warm, alkaline fracture water on the 17 Ledge of SURF. We hope to determine what groups of microbes inhabit the pool, and which groups can be isolated by culture-based methods. Additionally, we hope to isolate novel species of bacteria which may be valuable to medicine and biotechnology.

Materials & Methods

- Samples of water from a pool of fracture water on the 17 Ledge, 150 m from the astrobiology site at "Thiothrix Falls" on 1470 m level of SURF, were taken for direct plating onto 1/10 X R2A media[5] with 1% ATCC vitamins and fungicides (cyclohexamide and nystatin), pH 8.5.
- Samples of diluted pool water were placed in 6 diffusion chambers (1/50x R2A media, 0.1% vitamins, fungicides, pH 8.5, prepared in SURF drainage water), which were returned to the pool of water and left for 3 weeks.
- Agar from diffusion chambers was homogenized at BHSU, plated onto 1/10 x R2A media (see above), and left to incubate for one week. Colonies were transferred to slants of 1X R2A media (pH 8.5), gram stained, and tested for growth on Simmons Citrate agar and for catalase and cytochrome oxidase activity.
- To collect DNA from samples, microbes from the pool water were collected with a portable peristaltic pump and 0.2 µm pore cartridge filter, and samples of pool sediment and agar media from diffusion chambers were also obtained. DNA was extracted with MoBio PowerWater and PowerSoil kits. 16S rDNA libraries were prepared using an Illumina Nextera kit, and sequenced with an Illumina MiSeq device at the BHSU Center for conservation of Biological Resources (CCBR). 16S rDNA sequence data was analyzed using the Microbial Genomic module of CLC Bio (Qiagen, Inc.).
- DNA was extracted from bacterial isolates using a DNeasy Blood and Tissue kit (Qiagen). Randomly Amplified Polymorphic DNA (RAPD, "DNA fingerprinting") analysis[6] is being used to divide isolates into genotypic groups.

Results

- Pool sediment had abundant Acidobacteria, Chlorobi, Nitrospirae, Planctomycetes, and Proteobacteria (including *Acidiferrobacter*).
- Filtered pool water had mainly Proteobacteria, like *Thiobacillus*, *Methylococcus*, and *Methyloversatilis*, as well as some Nitrospirae and Planctomycetes.
- Agar from diffusion chambers had mainly Proteobacteria, especially *Hyphomicrobium* and *Pseudomonas*.
- We did phenotypic characterization of 100 bacterial isolates from direct plating of pool water onto 0.1X R2A media, and 100 other isolates from plating of media from diffusion chambers onto 0.1X R2A.
- RAPD analysis for genotyping of isolates is ongoing.

Collecting samples of water, sediment, and diffusion chambers from a pool on the 17 Ledge, 1470 m level, SURF.

Taxonomic Information for Charts to the Right

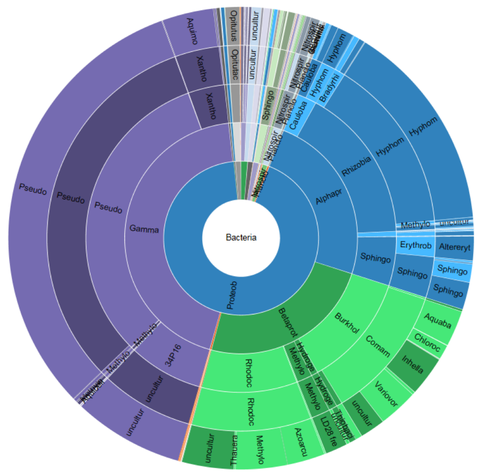
Phylum Key

Acidoba = *Acidobacteria*
Chlorobi = *Chlorobi*
Ectothio = *Ectothiorhodospiraceae*
Nitrospir = *Nitrospirae*
Plancto = *Planctomycetes*
Proteob = *Proteobacteria*

Genus Key

Acidiferr = *Acidiferrobacter*
Alphapr = *Alphaproteobacteria*
Aquimo = *Aquimonas*
Betaprot = *Betaproteobacteria*
Gamma = *Gammaproteobacteria*
Hyphom = *Hyphomicrobium*
Leptospi = *Leptospirillum*
Methylo = *Methyloversatilis*
Pseudo = *Pseudomonas*
Sphingo = *Sphingomonas*
Thiobaci = *Thiobacillus*
Variovor = *Variovorax*

Agar



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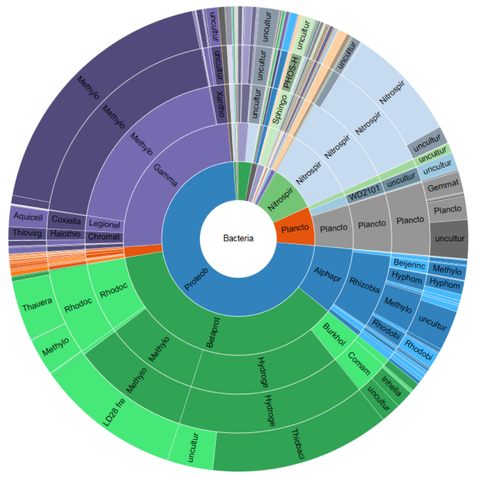
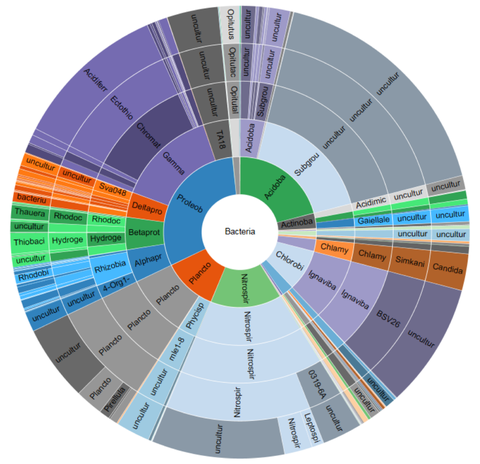


Sediment



Conclusions

- Microbial life is incredibly diverse in the 17 Ledge of SURF with potentially hundreds of different genera of prokaryotic organisms with widely differing metabolisms.
- *Acidiferrobacter*, which can oxidize ferrous iron or sulfide in warm, alkaline conditions, is common the pool sediment.
- Possible sulfide-oxidizing bacteria, like *Thiobacillus*; methane-oxidizers, like *Methylococcus*; and methanol oxidizers, like *Methyloversatilis*; are common in the pool water, which reveals that there are many different chemoautotrophic and chemoheterotrophic metabolic processes occurring in the pool water.
- Media in the diffusion chambers selected for chemoheterotrophs like *Pseudomonas* and *Hyphomicrobium*.
- It is currently unknown whether or not novel genera have been isolated from the pool by direct plating from water or plating media from diffusion chambers. When genotyping is complete, representatives of each genotype will be selected for PCR amplification of 16S rDNA and Sanger DNA sequencing to identify isolates.

References

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