Characterization of Microbial Life in Sanford Underground Research Facility Waters

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Introduction

The Sanford Underground Research Facility (SURF) is located in a complex former mine system that collects water originating from both the surface and deep underground aquifers. These waters pool in natural crevices and unused drifts, allowing for the formation of a wide variety of microbial ecosystems. These ecosystems can be characterized by the water chemistry and the microorganisms present in the pools.

Previous studies of SURF mine ecosystems have primarily focused on the composition of various biofilms which form on rock surfaces[1,6] or deep aquifer water. The aquifer contains unique groups of chemoautotrophic and chemoheterotrophic Bacteria and Archaea[4,5].

These communities are poorly understood, and many of the inhabitants of these waters have yet to be identified or cultured in a laboratory. Further research into these organisms could lead to the discovery of previously unknown microbes, whose unique metabolisms or products could have uses in medicine and industry. In order to gain a better understanding of important taxa and metabolic processes in each ecosystem, sampling was conducted at three different depths: 510 m, 1400 m, and 1500 m. These samples were filtered and sequenced in order to identify the microbial life present at each water pool.

Project Goal: identify microbes living in the waters of the Sanford Underground Research Facility in order to characterize these communities.

Materials and Methods

Sampling:

Samples of water from the following depths were taken and filtered using a MasterFlex E/S portable peristaltic pump using sterile silicon tubing through a 0.2 um Sterivex filter. Filters were frozen at -80 C until DNA extraction.

- **510 m Ellison Shaft Location:** a cavern with a bore hole funneling the water to lower levels. 15.3 L of water collected.
- **510 m Ellison Fan Location**: a cavern containing calcium deposits. Approximately 12 L was collected
- **1400 m Drift Location:** a flooded drift. 9 L of water collected.
- **1500 m Sump Location:** a sump where water is collected before being pumped to the surface. Sampling involved the collection of 12 L sump water in sterile containers and subsequent transport to the surface for pumping and filtering.

DNA Extraction and Purification:

DNA extraction was conducted using a DNeasy PowerLyzer Power Soil Kit (Qiagen) on the 510 m and the 1500 m samples and a DNeasy Power Water Kit (Qiagen) for each sample. On the samples originating from the 510 m and 1500 m levels there was significant buildup of sediment on the filter surface. This sediment was scraped off and run through the Power Soil Kit, while the filter itself was run through the Power Water Kit. There was negligible buildup on the 1400 m filter. The resulting elutions containing the sample DNA were futher purified using a Genomic Clean and Concentration Kit (Zymo Research)

Library Preparation and Sequencing:

DNA from each sample was diluted with PCR grade H_20 to a concentration of 5 ng/µL. PCR for each sample was performed to amplify the V4 region of the 16S rDNA sequence to detect bacteria and archaea taxa, and the V4 region of 18S rDNA sequence to detect eukaryotic taxa[5]. The recommended protocol for Illumina Dual-Indexing was followed[2], and the concentration of each sample was determined using a Qubit 2.0 fluorometer (Invitrogen). Each sample was then pooled and diluted to 5 pM for paried-end sequencing on an Illumina MiSeq system. Sequencing data was analyzed using CLC Bio Genomics Workbench and the Microbial Genomics add-on software (Qiagen).

Results: Prokaryotic Life

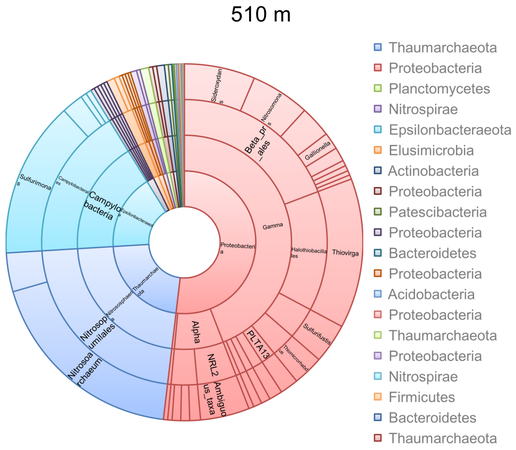


Figure 1: Combined 510 m Sunburst Diagram. This figure shows the relative abundance of the major inhabitants of the 510 m level.
The majority of the inhabitants at the 510 m level are Proteobacteria, primarily Gammaproteobacteria, which contain iron oxidizers, ammonia oxidizers, and sulfur oxidizers. There large numbers of Thaumarchaeota phylum members, which are generally ammonia oxidizers, and Epsilonbacteria, which present primarily as *Sulfurimonas,* sulfur and hydrogen oxidizers.

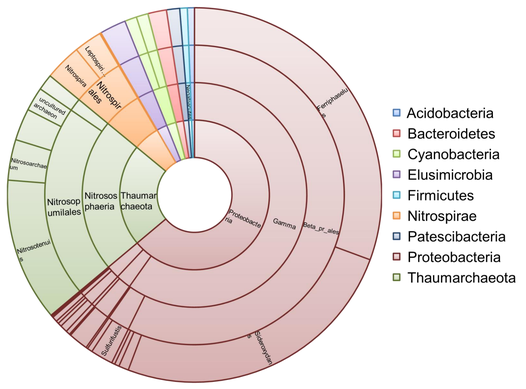


Figure 2 (left): 1400 m Prokaryotic Sunburst Diagram. This figure shows the differences between Gammaproteobacteria at the 1400 m and the 510 m; here, over half the prokaryotic components are iron-oxidizers. Another significant portion of these microbes are ammonia-oxidizing Thaumarchaeota.

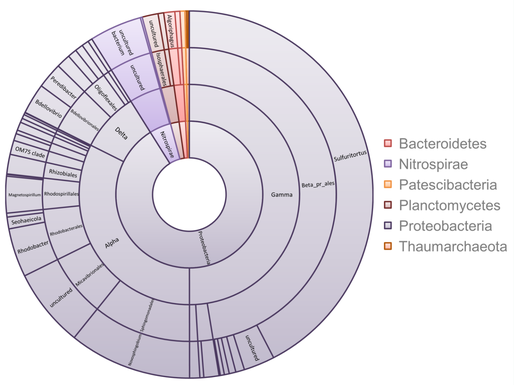
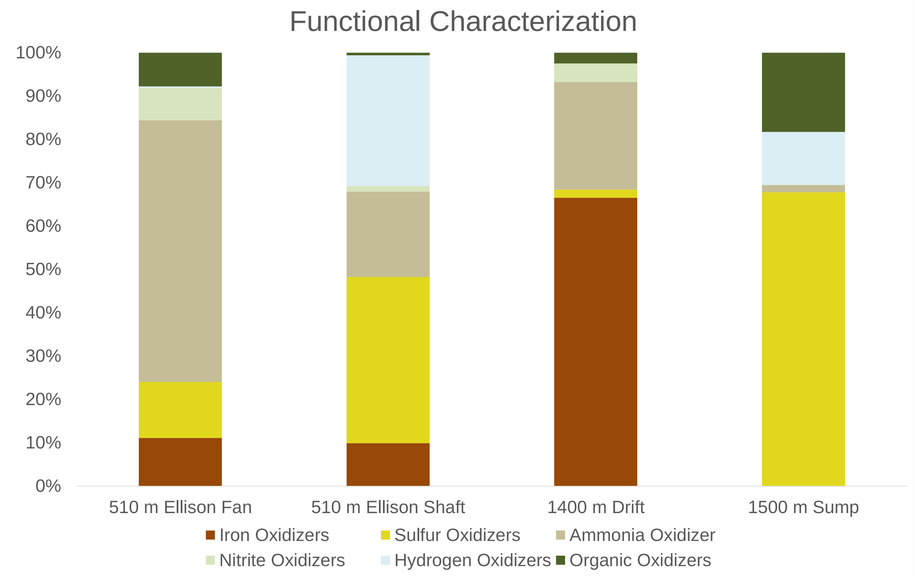
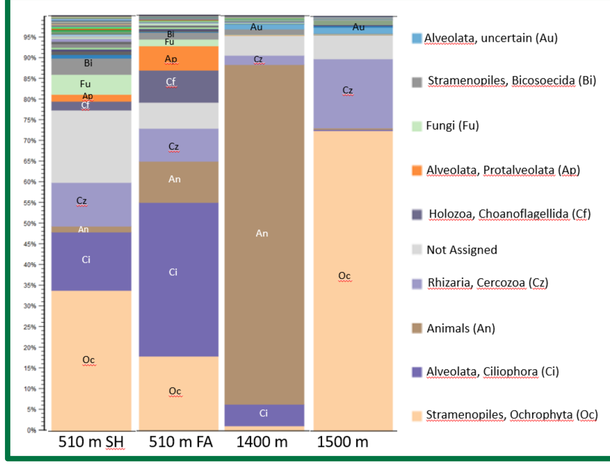


Figure 3 (above): 1500 m Prokaryotic Sunburst Diagram. This figure shows that the vast majority of prokaryotic life is Proteobacteria, primarily Gamma and Alpha-proteobacteria. A significant portion of these are *Sulfuritortus,* a genus which is capable of oxidizing a wide variety of reduced sulfur compounds.

Figure 4 (right): Functional Characterization of Prokaryotic Life. This permits the visualization of the different sources of energy of prokaryotes found at the individual sites. At the Ellison Fan site on the 510 m level there are primarily ammonia oxidizers (Thaumarchaeota and *Nitrosomonas sp.*), which differs from the 510 m Ellison Shaft site significantly. The latter is composed mainly of *Sulfurimonas sp.* and *Thiovirga sp.,* both of which oxidize sulfur and hydrogen. At the 1400 m level, there are primarily iron oxidizers (see Fig 2), and at the 1500 m there are primarily sulfur oxidizers (see Fig 3). Additionally of note is the fact that many of the bacteria using organic compounds for energy at the 1500 m are *Bdellovibrio* and related genera. These bacteria are parasitic and survive by invading and living in other bacteria.





Results: Eukaryotic Life

Figure 5 (left): Bar Chart of Eukaryotic Diversity. As expected, euakaryotic diversity was significantly lower than prokaryotic diversity.
Of particular interest are the Stramenopiles, specifically the group Ochrophyta present in large numbers at the 1500 m and the 510 m sites. Ochrophyta are typically photosynthetic organisms. The vast majority of these were identified as a previously uncultured genus of Chrysophyceae, a type of algae. These organisms are both phototrophs and heterotrophs. It can be inferred they are surviving off of dissolved organic substrates in the water.
At the 1500 m sump there were also large numbers of Cercozoa, specifically the genus *Metabolomonas* which are generally amoeboid protists. Absent at the 1500 but present in all other level were significant numbers of Ciliophora, particularly the Scuticociliates which are generally marine microbes.
At the 1400 m drift there was a large amount of 18S rDNA sequences associated with animals. Indeed, this DNA is associated with Triplonchida and Araeolamida, both nematodes, and Catenulida which is a flatworm.

Conclusions

- Microbial diversity seems to be related to depth: the greatest alpha diversity occurs at the 510 m level, while the lowest occurs at the 1500 m level. Each level and location seems to have some “dominant” type of metabolism for the bacteria present. This is likely related to the presence of these energy sources as either precipitate or dissolved in the water.
- These environments may be low-oxygen, particularly the 1400 m pool and the 510 m levels, due to the presence of iron oxidizers.
- Eukaryotic diversity is less than that of the prokaryotic, but this does exemplify that there is not just one type of eukaryote found in SURF waters- rather there are may types of protists and even multicellular organisms such as worms present.
- Future directions include direct plating of these waters onto media and the attempted cultivation of some of the many unknown/previously uncultured organisms found through the sequencing process. While these organisms may be difficult to culture in a lab setting, their ultimate discovery and further phenotypic characterization could prove useful in understanding deep underground microbial communities.

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