Exploration of the diversity of Eukaryotic, Prokaryotic and Archaea communities in deep subsurface microbial ecosystems

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♦ Introduction

Exploration of microbial diversity within unique ecosystems worldwide contributes greatly to our understanding of the complexity and diversity of life. Such exploration has revealed the presence of numerous novel lineages of Bacteria, Archaea, and Eukaryota, many of which are uncultured; has expanded our knowledge of the limits of life; and has provided insight to physiological and biogeochemical processes. Here we build on previous studies exploring the diversity of the deepest levels accessible within the Sanford Underground Research Facility (SURF). The characterization of these microbial communities and fungal bodies will allow us to have better insight to the diversity of microbes that have come to inhabit areas of this subsurface environment, and provide clues to the nature of life when it is shielded from cosmic radiation, and survives in carbon-poor extreme environments.

- Earlier studies of Bacteria and Archaea diversity present in samples of soil, water and soil-like biofilms sampled from SURF early upon re-entry indicated the presence of novel taxa[1, 2, 3].
- In 2011, geoscientists Borgonie and Onstott discovered the deepest living animal, *Halicephalobus mephisto* in a south African gold mine at depths ranging from 4224-11616 ft underground[4].

♦ Methods & Materials

ID of Fungi by sequencing of ITS region of rDNA:

- Fungal isolates were collected from various locations throughout the 4850 level at SURF and cultured in potato dextrose agar.
- PCR was used to amplify DNA with ITS1 and ITS4 primers using a Phire Plant Direct PCR Kit (ThermoFischer).
- The Applied Biosystems 3130xl genetic analyzer (Life Technologies) was used to perform Sanger sequencing on the amplified fungal DNA.
- The sequences were analyzed through Sequencher 5.2 (GeneCodes) and characterized using NCBI's nBLAST (nucleotide Basic Local Alignment Search Tool).

NextGen Sequencing of rDNA from Eukarya, Bacteria and Archaea within the Biofilms:

- Biofilms were collected from 4850 level of SURF. (Figure 1)
- DNA was extracted from the samples with the PowerLyzer PowerSoil DNA Isolation Kit.
- DNA was concentrated using a Zymo DNA Clean & Concentrator Kit.
- The 16S (Bacteria & Archaea) and 18S (Eukarya) rDNA from each biofilm was PCR amplified using Domain specific primers.
- The Metagenomic16S and 18S rDNA amplicon libraries were prepared for sequencing using the standard 16S Metagenomic Sequencing Preparation for the Illumina MiSeq system.
- Bacteria and Archaea 16S sequences were analyzed using the 16S analysis pipeline available in Illumina's BaseSpace System.
- Eukaryotic 18S sequences were analyzed through CLC Bio (Qiagen) for trimming and OTU clustering.
- nBLAST against GenBank (NCBI) was used to obtain the closest taxonomic matches for preliminary identification of diversity for each OTU.

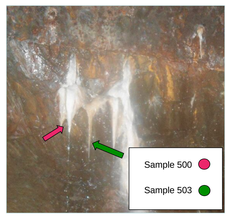


Figure 1. Two biofilms of a snottite-like texture differing in color were collected from a drift wall approximately 1 m from the drift floor. 500 appeared to be a light colored opaque white, while 503 had a brownish tinge. The biofilms were growing in an area where water was flowing from a drill hole that bathed these biofilms.

♦ Methods & Materials

Visual characterization:

- Regular light microscopy was used to scan small portions of the biofilm.
- Small portions of the biofilms were preserved for scanning electron microscopy (SEM) with a phosphate buffer containing 5% glutaraldehyde.
- The buffer was replaced by a series of ethanol washes with increasing concentrations until 100% has been reached.
- The sample was then placed under critical point drying followed by coating with a film of gold before placed under a scanning electron microscope.

♦ Results/Discussion

Fungi:

- Amongst the variety of genera detected, *Arthrobotrys* stood out as the most interesting. *Arthrobotrys* is a carnivorous fungi that feeds on nematodes by entrapping the roundworm with it's hyphae. (Figure 2)

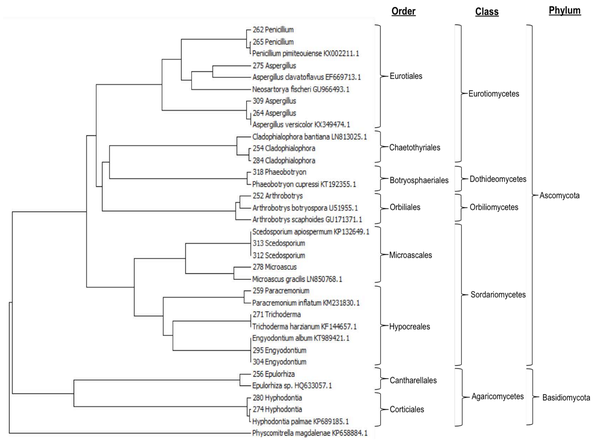


Figure 2 Phylogenic tree of fungal ITS sequences with reference sequences in GenBank. *Phycomitrella magdalenae* was selected as out-group to root the tree.

Bacterial life:
Sample 500

- 16S Metagenomics report indicates that the collected biofilms displayed a high abundance of Betaproteobacteria, Gammaproteobacteria and Bacteroidetes (Figure 3).

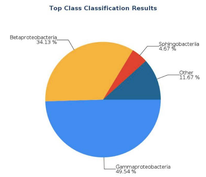


Figure 3 (Above) Pie chart of class abundance within sample 500.

Top Species Classification Results	BF4850-060216-500M
Classification	% Total Reads
Methylocaldum tepidum	47.25
Methylobacillus glycogenes	17.94
Runella limosa	2.63
Thiobacillus thioparus	2.26
Methyloversatilis universalis	2.25

Figure 4 (Above) Table representing the top 5 species present in sample 500.

- Amongst the species found with the highest abundance, it is possible that *Methylocaldum tepidum* (Gammaproteobacteria), a methane-oxidizing bacteria[3] *and Methylobacillus glycogenes (*Betaproteobacteria), a glycogen-producing bacteria utilize one another's metabolites. (Figure 4).
- Comparing previous studies performed at the Homestake mine, our results reveal some consistency with the most abundant Phylum identified in the soil sample from the Ross shaft wall studied by Waddell et al, 2010, but it differs significantly in abundance at the species level. The soil sample had a high abundance of aerobic sulfur-oxidizing bacteria of the *Thiobacillus*[1].

♦ Results/Discussion

Bacterial life:
Sample 503

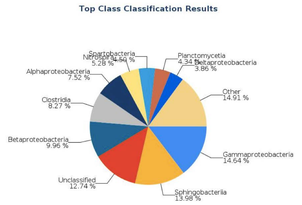


Figure 5 (Above) Pie chart of class abundance within sample 503.

Top Species Classification Results	BF4850-060216-503M
Classification	% Total Reads
Runella limosa	11.4
Methylocaldum tepidum	7.83
Clostridium papyrosolvens	3.09
Chondromyces pediculatus	2.26
Methylobacillus glycogenes	2.12

Figure 6 (Above) Table representing the top 5 species present in sample 500.

- 503 exhibited a much more diverse variety of bacterial classes with Gammaproteobacteria and Sphingobacteria being the most abundant.

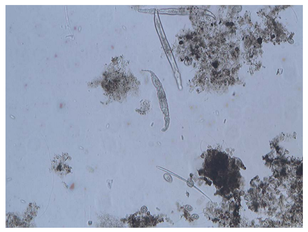
Archaea life:

- In contrast to the diversity found in the bacterial results, over 90% of the Archaea data were found to belong to the phylum Thaumarchaeota and grouped most closely to *Nitrosopumilus maritimus,* a common ammonia oxidizer that is usually found in non-coastal marine environments[5].

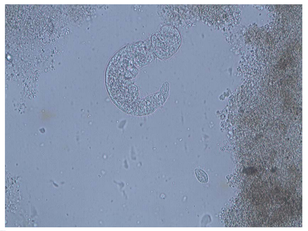
Eukayotic life:

- Contrary to common belief, the underground is not the desolate abyss that we imagine it to be, but rather a world blooming with microscopic life and communities that await further discovery.

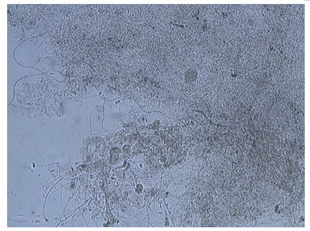
Light Microscopy



Sample 503 under Light Microscopy at 100X(Left) Multiple unidentified bilaterian and nematode.



Sample 500 under light microscopy at 400X (Right) Unidentified Eukaryotes.

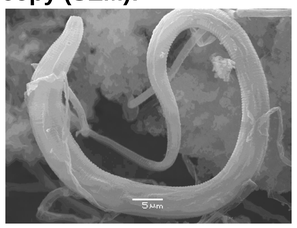


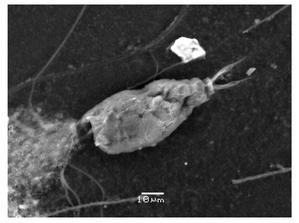
Sample 500 under light microscopy at 100X (Left) Filamentous biofilm with a variety of microbes.

Unidentified flagellate by light microscopy at 400X (Left) uses its single flagellum to propel itself forward and obtain sensory information.

Scanning Electron Microscopy (SEM):

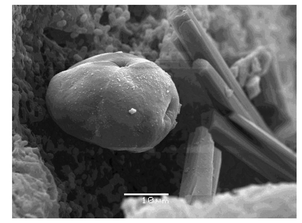
Nematode under SEM (Right)
2700X
presence supported by NextGen Metagenomic data that still awaits further analysis for specific genus.





Rotifer under SEM (Left)
2300X

Suspected pollen granule under SEM (Right)
2500X



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