(Fourteenth edition.) (pp. 447-460). Boston: Pearson

Discovering Biodiversity in Deep Sub-Terrestrial Ecosystems

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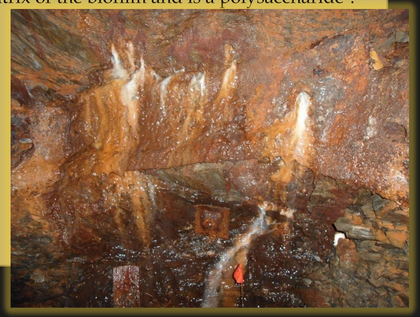
Introduction

Almost a mile under the earth in the tunnels of the old Homestake Mine, a thriving ecosystem of microorganisms resides despite the high temperatures, 100% humidity and the complete absence of cosmic radiation and light. These tiny creatures live in biofilms that grow on the mine walls in places where water is present. Biofilms are slimy substances that are composed of both microorganisms and extracellular polymeric substances (EPS). The EPS forms the primary matrix of the biofilm and is a polysaccharide[1].



Biofilm 500, taken by Beth Reman

Materials and Methods

Collection of the Biofilms

The biofilms were collected at the 17 ledge on the 4850′ level of SURF. The 17 ledge is always dark, the temperature averages 32°C, humidity is nearly 100%. and it smells vaguely of sulfur. Approximately 50mL of biofilm was collected in sterile conical tubes and taken back to the lab at ambient temperatures.

Microscopy

The biofilms were analyzed under a microscope to get an idea on what type of microscopic eukaryotes where present in each one. The microscope and software used was Olympus Bx60 and Olympus DP70 camera Image Manager respectively.

DNA Extraction

DNA from the biofilm was extracted using the DNeasy PowerLyzer Power Soil DNA Isolation Kit (Qiagen) according to manufacturer's protocol. All extractions where quantified by NanoDrop ND-100 Spectrophotometer.

PCR

Amplification was done using three primer sets. For the prokaryotes the 16S V3V4 region was amplified. For the eukaryotes the ITS primers ITS1 & ITS2 were used to amplify ITS1 and primers ITS1and 4 were used to amplify the ITS1:5.8S:ITS2 region of the ribosomal DNA[3].
The metagenomic Sequencing Library Preparation protocol from Illumina was used.

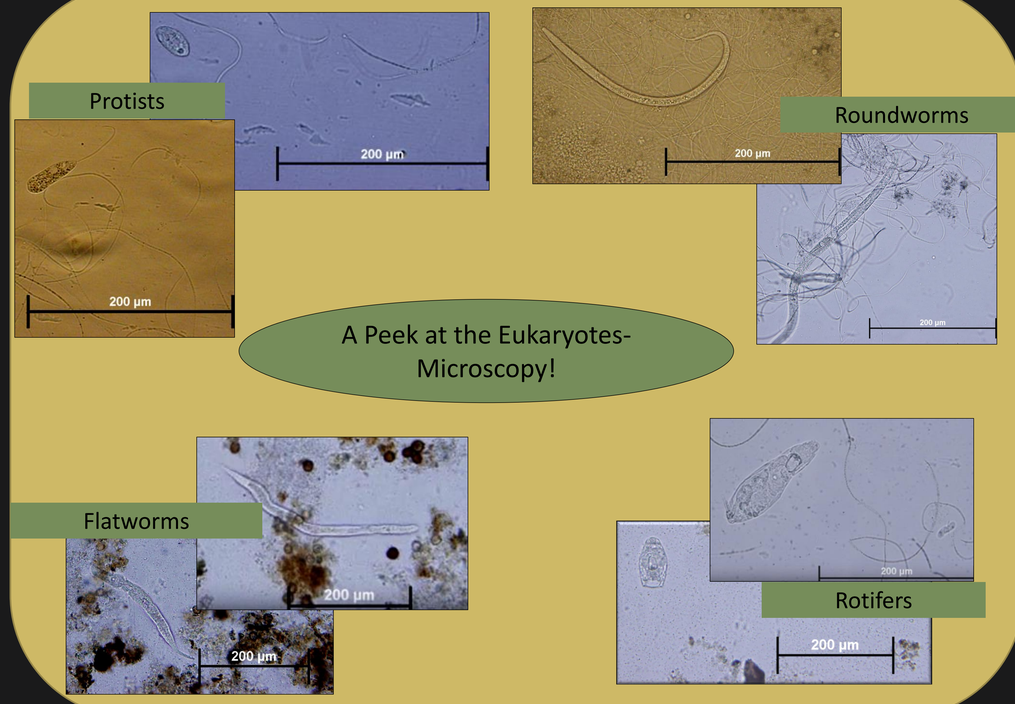
Next Generation Sequencing

The Illumina MiSeq high thouroughput system was used to sequence the amplified regions. Next Gen sequencing was completed by CCBR staff in house.

Data Analysis

CLC Bio version 10.1.1 software was used to analyze the sequence data. For the 16S analysis the Silva 16S v128 database was used at a 97% similarity level. For the fungal ITS analysis the Unite v7.1 database was used at a 97% similarity level.

A Peek at the Eukaryotes- Microscopy!



Sequencing Data

Most Abundant Genera of Bacteria (Figure 1)

Thiothrix (42%)
Methylococcus (14%)
Thiobacillus (4.3%)
Thiovirga (3.7%)
final 36% are unknown/Genera of 1%or less

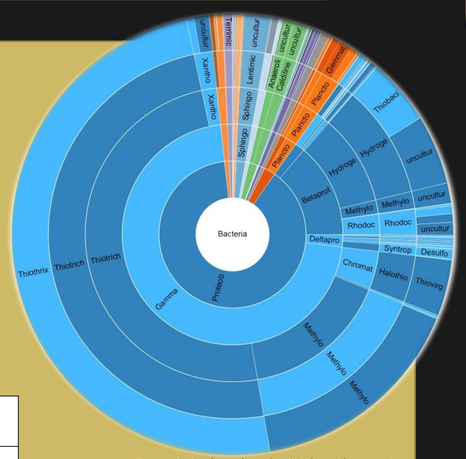


Figure 1. Analysis done by CLC bio. Alignment of 16S V3V4 sequences to Silva classified 86% of the sequences as bacteria. 14% were unidentified

Table 1. Four of the most abundant OTU's for the ITS primer.
Hits found by BLAST using the unpaired reads of the revers sequence from the ITS2 region. Average length 237 bp.

Name	Phylum	Accession Number	Percent Match
Naegleria lovaniensis	Heterolobosea	GU597044.1	99%
Vahlampfiidae sp.	Heterolobosea	KF547913.1	87%
Catenula macrura	Platyhelminthes	FJ384933.1	95%
Pentatrichomonas hominis	Parabasalia	KJ404270.1	82%

The Fungi Genera (Figure 2)

Trechispora(17.9%)
Hyphodontia(11.4%)
Unknown(18.81%)
Tapinella(7.81%)
Trichoderma(7.83%)
Sepula(5.21%)
Penicillium(4.62%)
Scytalidium(4.41%)
Peniophorella(4.34%)
Aphanocladium(2.08%)
Oidiodendron(1.89%)y
Guehomycis(1.66%)
Kockovaella(1.64%)
Amphinema(1.63%)
Naevala(1.63%)
Fellomyces(1.58%)
Pseudogymnoascus(1.25%)
Mortierella(1.22%)
Tranzscheliella(1.22%)
Amylocorticiellum(1.09%)
Endogone(0.78%)

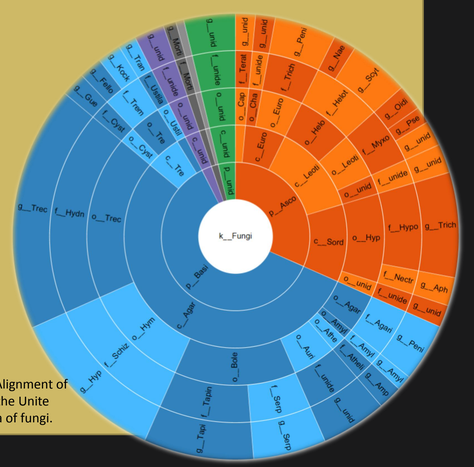


Figure 2. Analysis done on CLC Bio. Alignment of the ITS2 region from biofilm 500 to the Unite database classified to various genera of fungi.

Discussion

Bacteria

By briefly comparing biofilm 500 with data from the other biofilm sites on the 17 ledge gathered in previous experiments, it seems that many of the same orders may be found in the different biofilms. However, that's not to say that each site is the same. The genera represented in each biofilm and their proportions may be very different. Additionally, as this is further explored it may be the case that there are some major organismal differences in the different sites. This question has simply not been studied well enough yet to be sure.

The genera of bacteria found have some very interesting possible metabolisms and syntrophic relationships.
•Sulfate Reducers: *Desulfovibrio* and *Desulfomonile* (produce H_2S)[2]
•Sulfur Oxidizers: *Thiobacillus* and *Thiothrix* (oxidize H_2S to elemental sulfur)[2]
•Diazotrophs: phyla Chloroflexi, Chlorobi and Spirochates; Genus *Spirochaeta*[2]
•Nitrite Oxidizers: *Nitrospira*[2]
•Aerobic Methanotrophs: *Methylococcus* and *Methylocystis*[2]
•Ammonia Oxidizer: *Nitrosomonas*[2]

Fungi

Though sequencing produced fungal data, it is actually hard to say whether what was found is actively living in the biofilm or if some of the hits were from spores caught on the biofilms. However, the genera *Penicillium*, *Mortierella* and *Trichoderma* have been found growing on old timbers at the 48f0 level, as well as cultured from other biofilms found in the mine in previous experiments. It is likely the fungi entered the mine as spores via the ventilation system. The taxonomy found in the biofilm actually well represents what one might see in the forest. There is some evidence that fungi actually grow in the biofilms (Dr. Cynthia Anderson, personal communication). Further microscopy will be done to determine if they are growing in biofilm 500.

Microscopic Metazoans

As can be seen by the microscopy pictures, single celled protists and multicellular microscopic animals do live within the biofilm. Both ciliate and flagellate protists where seen along with nematodes, flatworms, roundworms and rotifers. They seem to be interacting well with the biofilm as possible nematode eggs were seen and a rotifer was witnessed feeding on either the biofilm itself or a fungus that may live in it.

Conclusion

The discovery of deep sub-terrain life is an ongoing project at SURF. In the future, primers for the 18S region of the DNA will be explored to further explore the eukaryotic diversity through next generation sequencing. In this investigation the aim is to find the best way of getting a true glimpse of the diversity of this amazing habitat. More specifically, primers that will specifically target metazoan eukaryotes is a desired discovery.

Acknowledgments

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NSF

Sanford Underground Research Facility

Citations

(1) Donlan, R.M. (2002). Biofilms: Microbial Life on Surfaces. *Emerging Infectious Diseases,* 8(9), 881-890. http://dx.doi.org/10.3201/eid0809.020063. Retrieved July 27, 2017.

(2) Madigan, M. T., Martinko, J. M., Bender, /k. S., Buckley, D. H., & Stahl, D. A. (2015). Chapter 14: Functional Diversity of Bacteria. *Brock Biology of Microorganisms* (Fourteenth edition) (pp.447-460). Boston: Pearson.

(3) White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., D. H. Gelfand, J.J. Sninsky, and T. J. White. Academic Press, Inc., New York.