Comparison of Deep Underground Biofilms and Surface Water Samples Using Next-Gen Sequencing

Patrick Noack[1], Beth Reman[2] , Shane Sarver[2]

1. Humboldt State University, 2. Department of Biology, Black Hills State University

Legend

- 4850 = Both biofilm samples from the 4850 ft. level of SURF.
- 4850-1 = the first biofilm sample collected
- 4850-2 = the second biofilm sample collected
- WC = Whitewood Creek

Introduction

The biosphere is now known to extend beneath the Earth's surface.[(1)]Gold mines in South Africa contain microorganisms, including Eukarya, that were trapped by stalactites. Organisms are found on multiple levels of Sanford Underground Research Facility (SURF) where water seeps through rock walls of tunnels. In SURF, this phenomenon happens from 800 ft. below to nearly a mile underground.

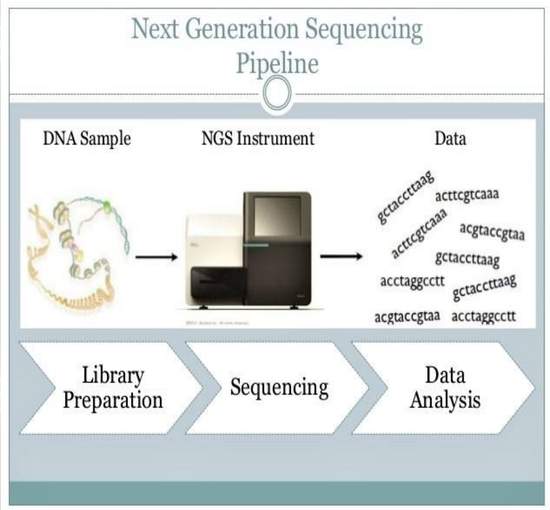
The purpose of this experiment is to compare eukaryotic organisms found underground to those in surface water. There is some evidence that surface water, such as WC, contributes to the water in SURF[(2)]. Here we used a combination of traditional microscopy and metagenomics to estimate taxonomic diversity.

Why Study the Subsurface Biosphere?

- Identifying novel species may lead to new or improved medicine.
- Studying underground communities advances our understanding of astrobiology.
- Studies such as this, provide a natural experiment to understand evolutionary processes.

Methods and Materials

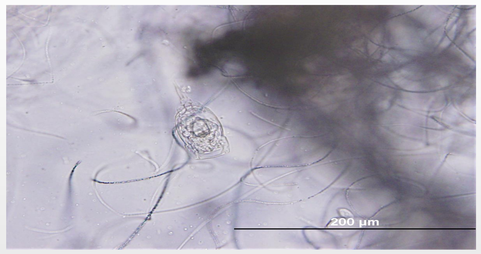
- Biolfims, soil and water filters collected June 2018
- Microscopy: Olympus B201, DP70 camera, DP70 Image Manager.
- DNA of the biofilm, soil, and filter samples extracted with Qiagen DNeasy PowerLyzer PowerSoil kit or DNeasy PowerWater kit.
- Dual indexing based on 16s Metagenomic Sequencing Library Preparation by Illumina.
- Sequenced on-site at BHSU with MiSeq.
- Data analysis: CLC Bio Workbench 11 and Microbial Genomics module.



Microscopy

Rotifer at 400x magnification from 4850-1

Nematodes at 400x magnification from 4850-2

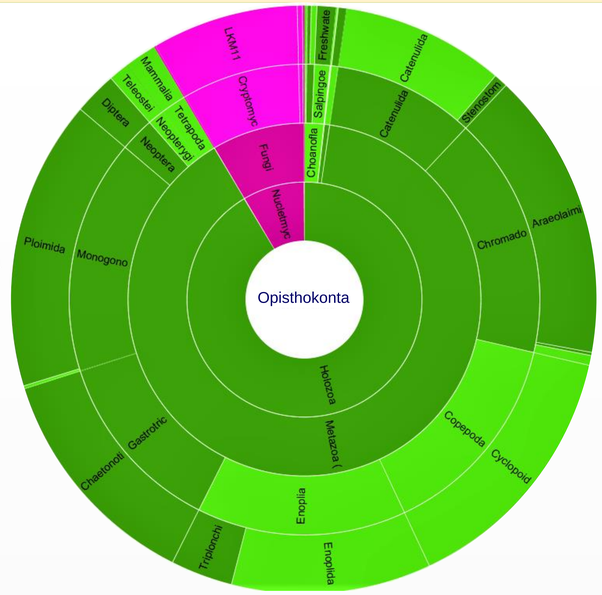


Rotifer at 400x magnification from 4850-2

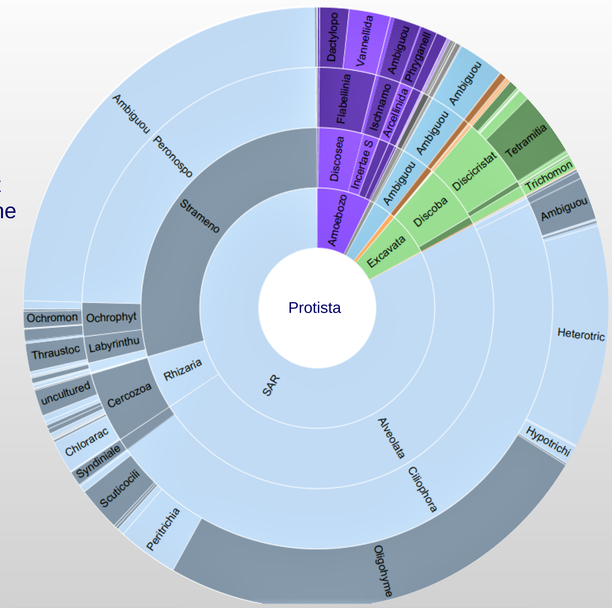
Amoeba and diatoms at 400x magnification from WC

Results – Underground Samples

Sunburst chart of Opisthokonta in the 4850 ft. level.

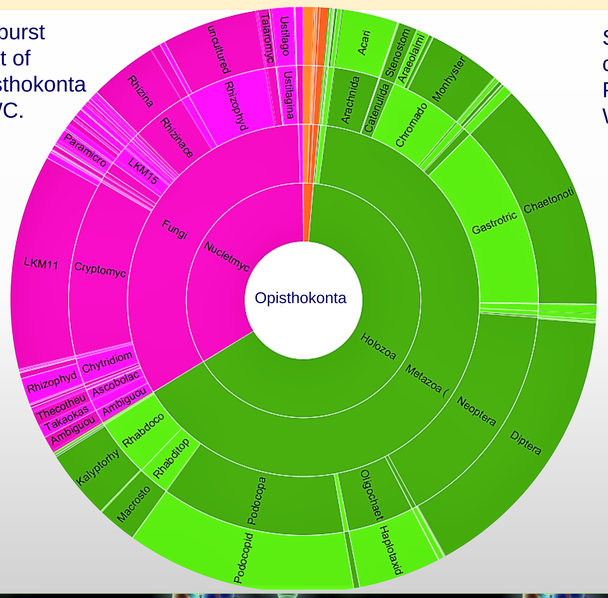


Sunburst chart of Protista in the 4850 ft. level.

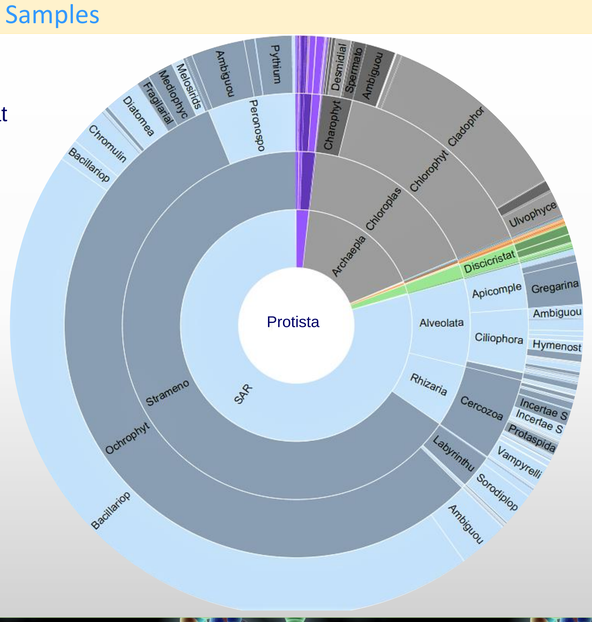


Results – Surface Samples

Sunburst chart of Opisthokonta at WC.



Sunburst chart of Protista at WC.



Discussion and Future Experiments

DNA sequences were processed and grouped into OTU's based on 97% similarity. These sequences were then compared with SILVA 18S v. 132 as a reference database. The OTU's that matched representative sequences in SILVA were then plotted as sunburst charts to visualize taxonomic diversity. The sunburst charts were divided into 2 major taxonomic groups (Opisthokonta and Protista) for simplicity.

Examination of OTU tables and sunburst charts indicate that some taxa are common to both underground and Whitewood Creek. For example, gastrotrichs (Phylum Gastrotricha) and some ciliate taxa (Phylum Ciliophora) are found at the 4850 level in SURF and in Whitewood Creek. On the other hand, a number of taxa identified in the underground samples are not found in Whitewood Creek. The amoebozoan protists (Phylum Amoebozoa), arthropods (Phylum Arthropoda), flatworms (Phylum Platyhelminthes), roundworms (Phylum Nematoda), rotifers (Phylum Rotifera), some ciliate taxa (Phylum Ciliophora) and heterokonts (Phylum Heterokontophyta) are found primarily in 4850 samples.

Research is ongoing to understand the biological diversity and evolution of the eukaryotic organisms found at the 4850 level in SURF.

Collecting the 4850-2 sample

References

[(1)]Borgonie, Gaëtan, et al. "Deep Subsurface Mine Stalactites Trap Endemic Fis Sure Fluid Archaea, Bacteria and Nematoda Possibly Originating from Ancient Seas." Frontiers in Microbiology, vol. 6, no. JUL, 2015, doi:10.3389/fmicb.2015.00833.

[(2)]Rahn, Perry H., and William M. Roggenthen. Hydrogeology of the Homestake Mine. Vol. 81, 2002, pp. 19–25.

Background picture acquired from cssanalytics.wordpress.com

Acknowledgements

Thanks to Sanford Underground Research Facility (SURF) for the accommodations and safety training.

Thanks to Tom Regan , Luke Scott, technicians and maintenance crew for assisting us at SURF.

Funding provided by NSF/REU 1560474 .

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.