Microbial diversity of rock surfaces on the 244 m level of the Sanford Underground Research Facility at different spatial scales

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Introduction

Microbes are the most abundant and diverse life forms on Earth, and the majority remain undiscovered. They are widely used in bioremediation, biotechnology, and pharmacology. The Sanford Underground Research Facility (SURF), located in the former Homestake mine, has unique microbes on rock surfaces, including thin, whitish, “cave silver” biofilms with abundant Actinobacteria (1). We collected small-scale samples from 244 m level at SURF, an area that has been mostly undisturbed since mining in the 1940s. Regions adjacent to cave silver biofilms were sampled, and16S rRNA gene sequencing was used to examine microbial diversity at different spatial scales, ranging from 1 mm to 50 m.

Methods

1. Samples of rock surface microbes were collected for 16S rRNA gene sequencing from five sites on the 244 m level of SURF (Figure 1). The sites were 10 m apart. Each site had five subsites, 20 to 100 cm apart. At each subsite, five samples, each 4 to 20 mm from each other, were collected by pressing micropipette tips to the rock wall surface. Rock sediment particles containing microbial cells adhered to the micropipette tip and were used for sampling microbial DNA.
2. Microbial cells on particles were mixed with lysis buffer (2) and lysed by freeze-thaw cycles. Direct Polymerase Chain Reaction (PCR) of the lysate using 2x KAPA HiFi HotStart ReadyMix DNA polymerase (2) was used to amplify the V4-V5 region of the 16S rRNA gene, and indices were attached using a Nextera kit (3) to create a gene library for sequencing.
3. Libraries were quantified using a Qubit fluorimeter and sequenced with an Illumina NexSeq DNA sequencer.
4. DNA sequences were analyzed using the CLC Microbial Genomics Workbench software (4), and the Operational Taxonomic Units (OTUs) were determined using the SILVA 138 database.

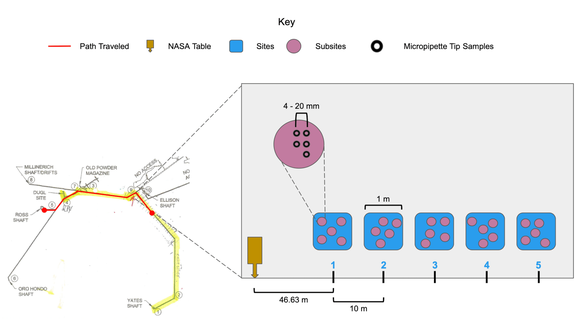

Figure 1: Map of the 244 m level, and a diagram of sample collection sites.

Results

All sites are in the Poorman Formation and have foliated rocks containing phyllite. Sites 3, 4, and 5 had water previously flowing over the rock surface while Sites 1 and 2 were drier and crumblier.

There was great variation in the number of sequencing reads per sample, ranging from less than 10,000 to over a million. Samples with fewer than 100,000 reads were not included in subsequent data analysis because rare OTUs may not be represented in samples with low reads.

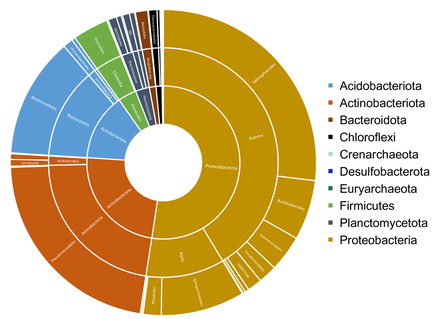

Figure 2: Sunburst depicting abundance of different taxa from 16S rRNA gene sequencing of all samples. Abundance of taxa is represented in the order (from inside out): phylum, class, and order.

Approximately 50% of the estimated reads were Proteobacteria, and ~50% of the Proteobacteria was identified as Salinisphaerales. Actinobacteriota, largely comprised of Pseudonocardiales, makes up ~25% of reads. Other prominent phyla include Acidobacteriota, namely Blastocatellales, and Chloroflexi at less than 25% of all OTUs.

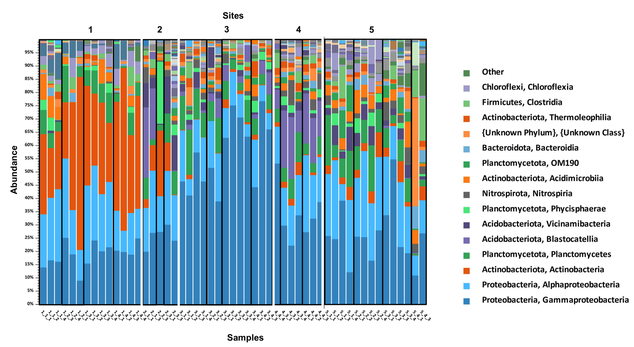

Figure 3: Bar graph portraying abundance of different phyla and classes from different samples and five sites.

There are similarities in composition of taxa within sites; for example, there is a high proportion of Actinobacteria in Site 1 and a high proportion of Gammaproteobacteria in Site 3 .

Phylum	Class	Order	Family	Genus	% Reads
Acidobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Crossiella*	13.908
Acidobacteriota	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	*Panacagrimonas*	13.724
Acidobacteriota	Blastocatellia	Blastocatellales	Blastocatellaceae	*JGI 0001001-H03*	12.752
Acidobacteriota	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	*NA*	11.674
Acidobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	8.0097
Acidobacteriota	Gammaproteobacteria	Burkholderiales	Sutterellaceae	*Uncultured*	4.3148
Actinobacteriota	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Lysobacter*	3.8443
Actinobacteriota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Polymorphobacter*	2.8251
Actinobacteriota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	2.6098
Actinobacteriota	Nitrospirales	Nitrospirales	Nitrospiraceae	*Nitrospira*	2.0733

Table 1: Most common genera (> 2% of reads) present in sample set used in 16S rRNA gene sequencing.

Common genera included *Pseudonocardia, Crossiella, Panacagrimonas, Sphingomonas, Polymorphobacter,* and *Lysobacter.*

Figure 4: Chao 1 bias-corrected index to determine alpha diversity of samples from 16S rRNA gene sequencing.

The Chao 1 bias-corrected index has several lines that do not plateau, indicating those samples do not have all possible OTUs represented, most likely because of few sequence reads.

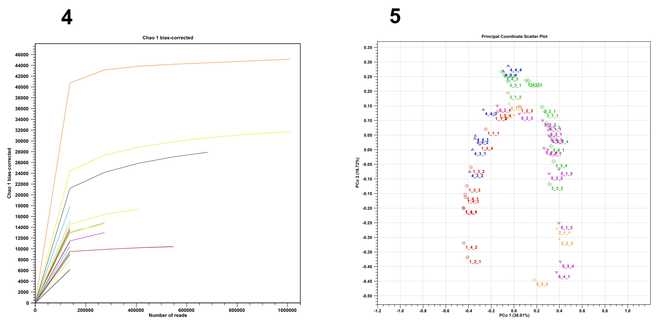

Figure 5: Principal Coordinate Analysis to illustrate beta diversity of 16S rRNA gene sequencing samples.

Principal Coordinate Analysis shows clustering of samples within sites but not subsites

Discussion

Conclusions

Although we were able to use micropipette tip sampling to examine the abundance of common taxa at a millimeter scale, there were difficulties in the PCR amplification of the 16S rRNA genes which led to variable and sometimes low number of sequencing reads for some samples. Hence, we were not able to accurately compare species (OTU) richness among samples.

We had hypothesized that rock sediment samples within a subsite (within 2 cm of each other) would have greater similarity in microbial taxa than those from different subsites. However, this was not observed, yet samples from the same site (within 1 m of each other) had a similar abundance of microbial taxa.

Next Steps

- Modifying DNA extraction, PCR protocols, and 16S rRNA gene library preparation to obtain more consistent numbers of DNA sequencing reads, so the data will be more representative of OTUs present.
- Further investigating the geology of the 244 m level and how it impacts microbes.
- Isolation of microbes using culture-based techniques and phenotypic and genomic characterization of isolates.

References

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