Development of "Cave Silver" Biofilms on Artificial Rock Surfaces in the Sanford Underground Research Facility

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Introduction

Due to the unique conditions found at the Sanford Underground Research Facility (SURF), this former gold mine is home to an extensive number of experiments spanning various fields of science.
The "17 Ledge" is an area of SURF that is 1470 meters below the surface. It is a warm and humid region where extensive "cave silver" biofilms form on rock walls. These biofilms, containing Actinobacteria, Proteobacteria, and other groups, are superficially similar to the biofilms we collected from the cave walls (Pasic et al. 2010, Lavoie et al. 2017). In this study, sterile rock slabs were attached to poles in the "17 Ledge" and left over a period of time to allow bacteria to colonize.
We hypothesized that the number of bacterial cells and species diversity would increase exponentially with time. We also hypothesized that the composition of the recently sampled communities would resemble that of mature cave silver biofilms.

Methods

Fig 1. Four poles with plastic petri dishes and sterile rock slabs attached

- Four poles were placed 100 feet from "Thiothrix Falls" at the "17 Ledge" with four sterile rock slabs attached to each pole. These samples are collected from the area three separate times: fifteen days, forty days, and sixty days after being initially placed underground. They were then taken back the lab where a 10 cm^2 area was swabbed and placed in 5mL of sterile tap water. Serial dilutions were made in 9 mL tubes of sterile tap water. 0.1 mL of these dilutions were plated out onto 0.1X R2B sediment extract plates containing fungicides.
- After a 10-day incubation period at 30°C, we counted colonies on plates and ran a number of different phenotypic tests on the isolates: gram staining, scanning electron microscopy, inoculating in R2B and glucose phenol red, and streaking on Starch and Simmons Citrate agar plates.
- In order to extract microbial DNA, we scraped a 10 cm^2 area with a small sterile spatula to put into a sterile 2mL tube containing 1mL of sterile phosphate-buffered saline (PBS). The supernatant was then removed, leaving the sediment pellets in the tube for DNA extraction.
- After extracting DNA with a MoBio PowerSoil kit, we prepared a library of V4 16S ribosomal RNA gene amplicons, which were then sequenced with an Illumina MiSeq System. We used three samples from each site with the highest concentration of DNA.
- The data accumulated from the Illumina MiSeq System was analyzed using the CLC Genomics Workbench 11.

Results

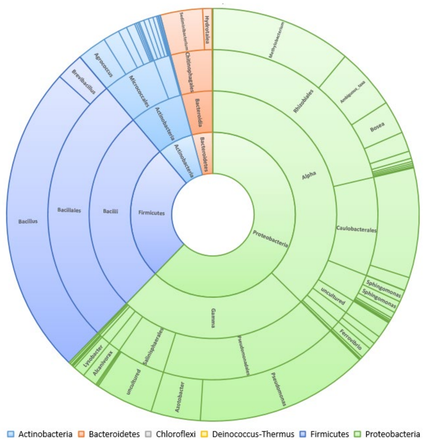

Fig 2. Microbial diversity after rock slab has been underground for fifteen days

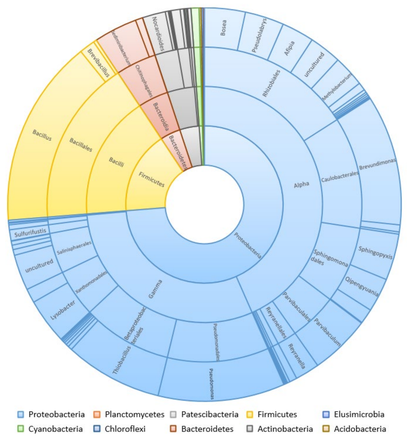

Fig 3. Microbial diversity after rock slab has been underground for forty days

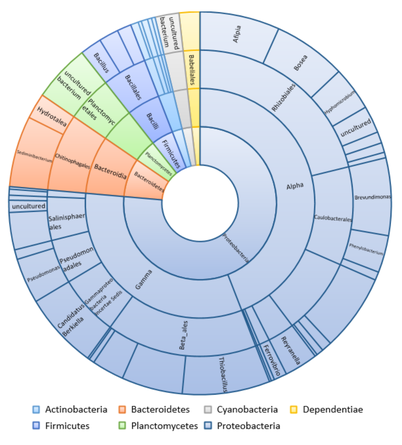

Fig 4. Microbial diversity after rock slab has been underground for sixty days

Fig 5. Microbial diversity of mature cave silver (Thompson et al., 2019)

- The above figures (fig. 2–5) display the composition of how the underground biofilm developed over time. The newer underground rock slab samples have some genera in common with the mature cave silver sample. The major phyla of bacteria beginning at the fifteen day samples are Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. One of the most significant groups of Proteobacteria are *Pseudomonas*, which has the ability to develop biofilms (Rasamiravaka et al. 2015). Proteobacteria make up a large percentage of the fifteen day composition, and it increases as time wears on. This trend occurs at the expense of Firmicutes, mainly *Bacillus*. However, it is still considered a major fraction of each composition. Additional groups start to appear, such as Chloroflexi and Acidobacteria, as the amount of time a rock slab spent underground increases. Planctomycetes is a group that begins to show up at the forty day samples. Another group that appears as the samples get more mature is Cyanobacteria. However, Actinobacteria do not become more numerous from fifteen to sixty days, even though they are abundant in mature cave silver (fig. 6).
- Figure 6 displays the mean and standard error of colony forming units per centimeter squared (CFU/cm^2). Although bacteria colonized on the rocks within fifteen days, the number of cultivable bacteria did not increase from fifteen to sixty days.
- Figure 7 shows the number of predicted species (operational taxonomic units or OTUs) over time using Chao 1. The increase in species abundance stops at forty days.
- Some of the variation in the composition of bacterial communities was observed among rock slabs colonized for the same number of days (fig. 8).

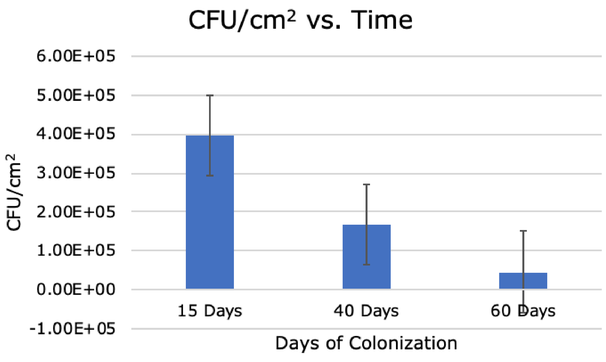

Fig 6. Quantification of colony forming units over time

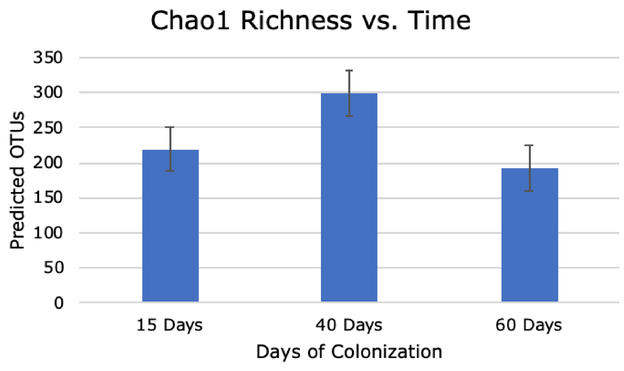

Fig 7. Quantification of Chao1 values over time

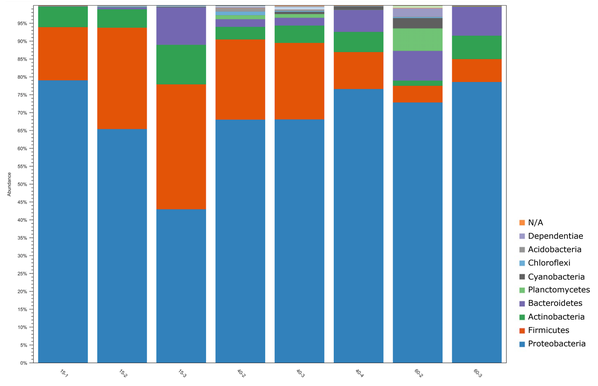

Fig 8. Comparison of the Microbial Diversity of all rock samples over time

Conclusions

It is possible that the condensation on the rock slabs underground could be washing away some bacterial cells, which would account for the decreasing trend of colony forming units from fifteen to sixty days.

Bacillus and Pseudomonas appear as early colonies on the underground rocks at SURF. They are replaced by other groups of bacteria with time.

A mature cave silver community appears to take much longer than sixty days to develop.

References

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