Isolation of Bacteria from "Cave Silver" Biofilms in the Sanford Underground Research Facility, Lead, SD

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Introduction

The Sanford Underground Research Facility (SURF) supports diverse microbial communities in sediments, fracture water, and biofilms on surfaces. These include sulfate reducing chemohetertrophs and chemoautotrophic sulfide and nitrite oxidizers in biofilms and sediments (Waddell et al. 2010) Whitish, iridescent "cave silver" biofilms thrive on the 17 Ledge of the 4850' level of SURF, an area characterized by 32º C heat and humidity near 100%. These cave silver biofilms superficially resemble iridescent biofilms found in limestone caves in Europe. Actinobacteria, Alpha-Proteobacteria, and Acidobacteria are abundant in silver biofilms in Europe and in SURF, but the species in SURF appear to be different than those in Europe (Pasic et al. 2010, Thompson and Bergmann 2016). Actinobacteria have possible uses in industry and medicine, including antibiotic production, but can be difficult to isolate, often requiring special media or isolation techniques (Velikonja et al. 2014). Here, we describe the isolation of bacteria from cave silver biofilms at SURF, using low nutrient media.

Figure 1. Cave Silver growing on the wall of a drift on the 17 ledge. Cave silver biofilms are light reflecting, and grow on sediment covering the rock face in the most humid area of the tunnel.

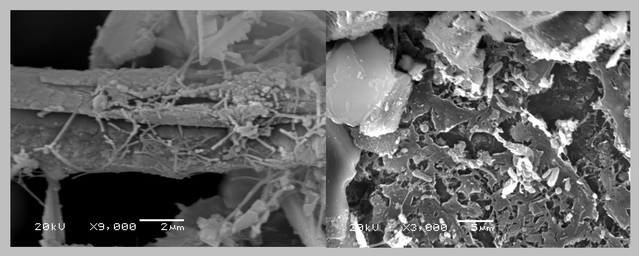

Figure 2a,b.SEM images from cave silver sample. (a) Filamentous bacteria covering an inorganic particle along with smaller bacterial cells. (b) Small rods and cocci. and cocci.

Methods

- **A 0.207g cave silver sample was collected from the 17 Ledge, plated onto low-nutrient gellan gum media (0.1X R2B with 1% ATCC vitamin supplement and fungicides), and incubated at 30°C for 10 days.**
- **Individual colonies were picked and streaked onto R2B agar media in order to isolate pure colonies.**
- **DNA was extracted from the bacterial isolates using Qiagen DNeasy kits.**
- **Randomly Amplified Polymorphic DNA PCR with the primers BOXAR1, followed by agarose gel electrophoresis, was used for DNA fingerprinting of isolates to determine how many Operational Taxonomic Units (OTUs) were present (Passari et al. 2015).**
- **Samples of cave silver were also fixed in phosphate buffer with 2.5% glutaraldehyde, dehydrated, critical point dried, sputter coated with gold, and viewed under a scanning electron microscope (SEM).**

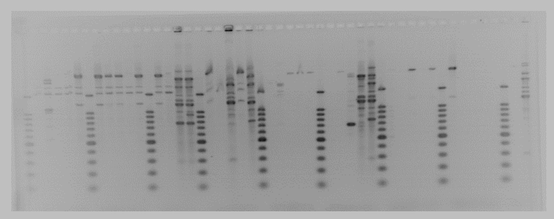

Figure 3a.

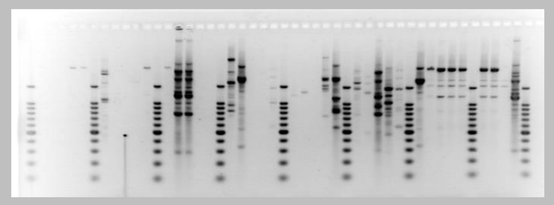

Figure 3b.

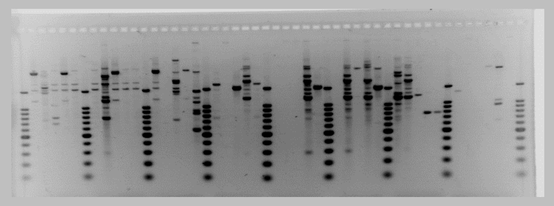

Figure 3c.

Figures 3a-c. Images of each of the 120 samples of amplified DNA on electrophoresis gels. Approximately 20% of the DNA samples did not amplify. A total of 29 OTUs were identified form this data.

Results

OTU	# of Samples	Gram +/-	Morphology	Base Pair Length
1	18	+	0.4µm diameter cocci among mycelium	1900, 1750, 1550, 1250
2	8	+	0.5µm diameter filamentous cells, 0.2µm cocci	1750, 1550
4	5	+	0.1µm diameter cocci	1600, 750
5	11	+	0.2µm diameter cocci	1900
8	5	+	0.3µm diameter cocci	2000, 1800, 1600, 1250
10	3	-	0.2-0.4µm length ellipsoid	2000, 1250
13	3	+	0.2µm diameter cocci	1700, 1500
15	4	+	1µm length ellipsoid	1900, 1650, 1550

Table 1. Final morphological, gram staining, and DNA fragmentation data for cave silver isolates After PCR and electrophoresis, a total of 29 OTUs were observed from the 120 isolates. Approximately 65% of the isolates were gram positive, and 35% gram negative. Many isolates has a mycelial growth form, indicating possible Actinobacteria, while many others were gram-positive cocci.

Discussion and Conclusion

The goal of this research was to isolate as many species of bacteria as possible from a sample of cave silver collected from SURF. The initial 120 isolates resulted in 29 OTUs and 6.714810*10^6 colony forming units. However, after completing a round of RAPD PCR on all isolates, about 20% of the samples did not amplify and yielded no data. Despite this problem, we still isolated a fait amount of bacterial species. Future work will involve repeating PCR to obtain amplified DNA from all isolates, and genotypic characterization of all isolates with random amplified polymorphic DNA analysis and 16s rDNA sequencing.

Acknowledgements

We would like to thank Amanpreet Brar, Ethan Thompson, Jesse Larson, and Oxana Gorbatenko for their help with various aspects of this project

References

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