

MICROBIAL COMMUNITIES OF ICELANDIC LAVA CAVES

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Abstract

Iceland is a country with many great Holocene lava caves that contain a diversity of microbial deposits. Our study examined a variety of microbial communities that included microbial mats, snottites, organic ooze, mineral deposits, and surface soils above the caves. The samples varied in color from white to tan, yellow/gold, orange, to the brown of surface soils. Four caves were sampled: Þríhnúkagígur, Leiðarendi, Raufarhólshellir, and Vatnshellir. Scanning electron microscopy (SEM) revealed the presence of biofilm, filaments, rods, and clusters of coccoid shapes, plus evidence of iron and sulfur minerals. DNA analysis demonstrated that the surface soils above the two of the caves are more diverse and substantially different from cave samples. All samples contained Acidobacteria and Proteobacteria, but surface soils were the only samples to contain a new candidate phylum, AD3. The snottites

were more similar to microbial mats than expected and were not acidic as the snottites are in sulfur caves around the world. While the microbial mats were fairly similar in composition at the phylum level, the mineral deposits varied from sample to sample. Overall, the type of sample (mat versus mineral versus surface soil) made the greatest difference in composition.

Methods

Site descriptions and sample collection:

In 2013, we have sampled four lava caves (Þríhnúkagígur, Leiðarendi, Raufarhólshellir, and Vatnshellir) for microbial communities for scanning electron microscopy (SEM) analysis and DNA sequencing. Þríhnúkagígur is a 4000 years old (tephrochronological dating) open vertical volcanic conduit (OVVC). The crater reaches down to a depth of 201 m and is descended on a platform (Fig. 1). Samples were taken at 120-130 m depth from a 20-30.000 years

old tuya base. Leiðarendi is a little over 1000 m long and 2000 years old (tephrochronological dating). Leiðarendi has become the most frequented cave in Iceland with 50-100 visitors per day on the average. Raufarhólshellir is 5600 years old lava cave (carbon dating), 1360 m long, wide (10 m on average). Vatnshellir is 8-10,000 years old (sea level 15-20 m lower than present). Vatnshellir is special because it is on three levels. The cave reaches down to a depth of -36 m, with a total length of 200 m.

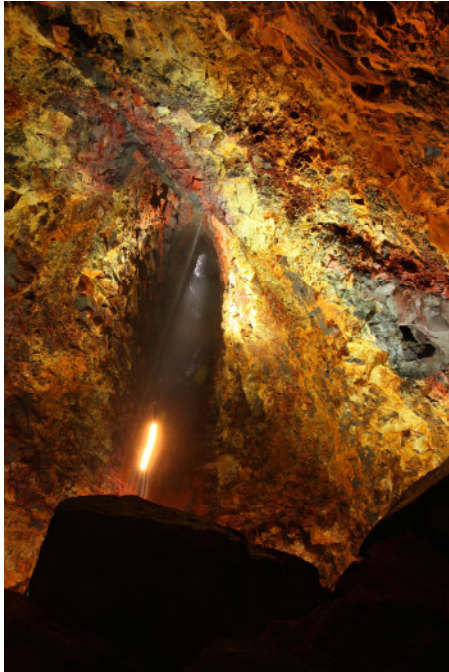


Figure 1: Overview of the descending platform used to enter Þríhnúkagígur. Photo by K. Ingham.



Figure 2: Gunnhildur in Leiðarendi. Photo by K. Ingham.



Figure 3: Gunnhildur in Raufarhólshellir. Photo by K. Ingham.



Figure 4: Gunnhildur in Vatnshellir. Photo by K. Ingham.

Samples were taken aseptically using a flame-sterilized cold chisel, and were immediately covered with sucrose lysis buffer to preserve the DNA (Giovannoni et al. 1990). Rock chips with microbial mats or mineral deposits sampled for SEM were mounted directly on SEM sample stubs in the field.

Scanning electron microscopy

Samples were air dried, and coated with Au-Pd metal for imaging in the laboratory. They were then examined on a JEOL 5800 SEM equipped with an energy dispersive X-ray analyzer (EDX), at high vacuum with an accelerating voltage of 15KeV with a beam current between 0.1 to 0.01 nA.

Results and Discussion

SEM

Scanning electron microscopy (SEM) revealed that the yellow crumbly mineral deposit of Þríhnúkagígur contained abundant biofilm with a plethora of extruding filaments (Fig. 5) and clusters of round, coccoid shapes with short hair-like extensions. The white microbial mat from Leidarendi contained an abundance of fuzzy coccid shaped morphologies (Fig. 6), biofilm, some of which had many rod-shaped morphologies embedded in it, and some filaments. The hard, gold-colored mineral deposit from Raufarhólshellir showed biofilm covering mineral crystals, some of which contained iron oxides and sulfur minerals. The sulfur deposit from Vatnshellir contained some beautifully folded biofilm (Fig. 7).

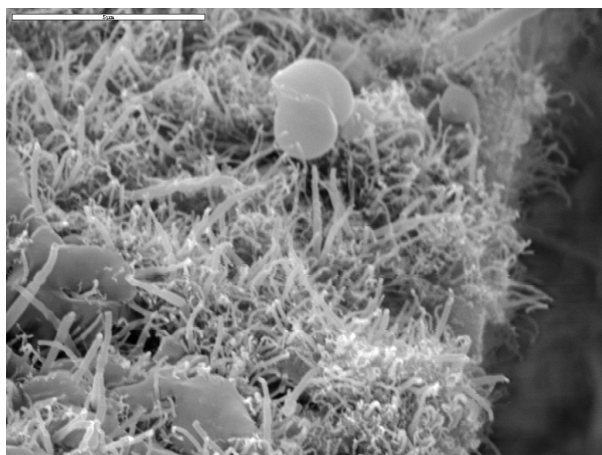


Figure 5: Overview of filament like structures on a sample of yellow, crumbly mineral deposit in Þríhnúkagígur.

DNA sequencing

The Actinobacteria, which give caves their musty odor, were more abundant in microbial mats, with the exception of one white microbial mat, and surprisingly were not very abundant in surface soils. Actinobacteria were also not very abundant in mineral deposits, or in the snottite or ooze samples (Fig. 8). Another abundant

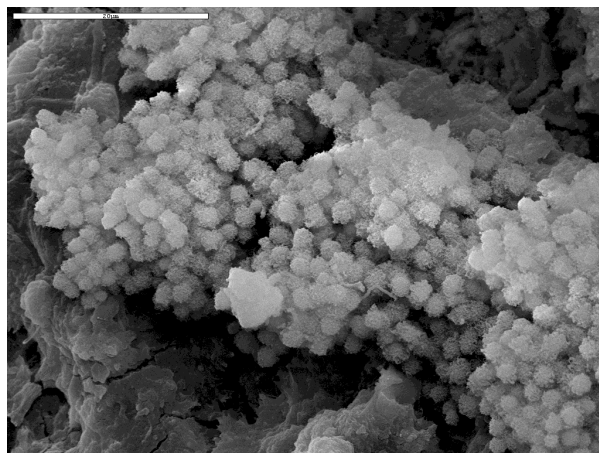


Figure 6: Clusters of coccoid shapes with short hair-like extensions from the white microbial mat in Leidarendi.

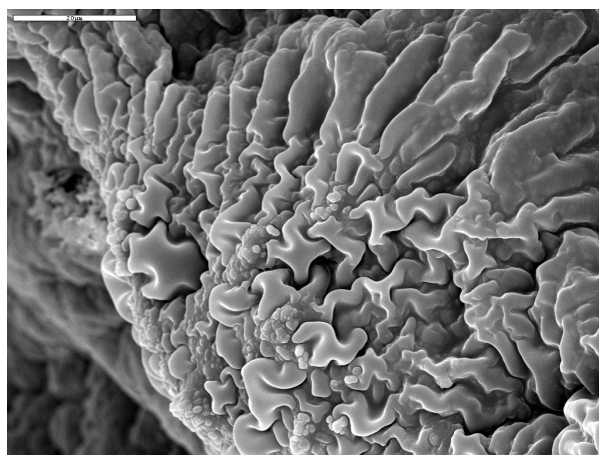


Figure 7: Folded biofilm from a sulfur deposit in Vatnshellir.

group in most samples was the Acidobacteria, most of which were undescribed, uncultured bacteria. Gammaproteobacteria, a class that contains some of the acidophilic bacteria found in snottites in sulfur caves, was not very abundant in the snottite found in Leidarendi, or in the ooze sample or in surface soil samples. Also interesting was the occurrence of the candidate phylum NC10, which was discovered in the Nullarbor Plain caves of Australia. NC10, a methanotroph that reduces nitrite (Ettwig et al. 2009), was mostly found in the mineral deposits sampled.

This parallels several of the findings of Hathaway et al. 2014, who studied Hawaiian and Azorean lava cave microbial mats. A notable exception is the lack of Nitrospirae in the Icelandic lava caves.

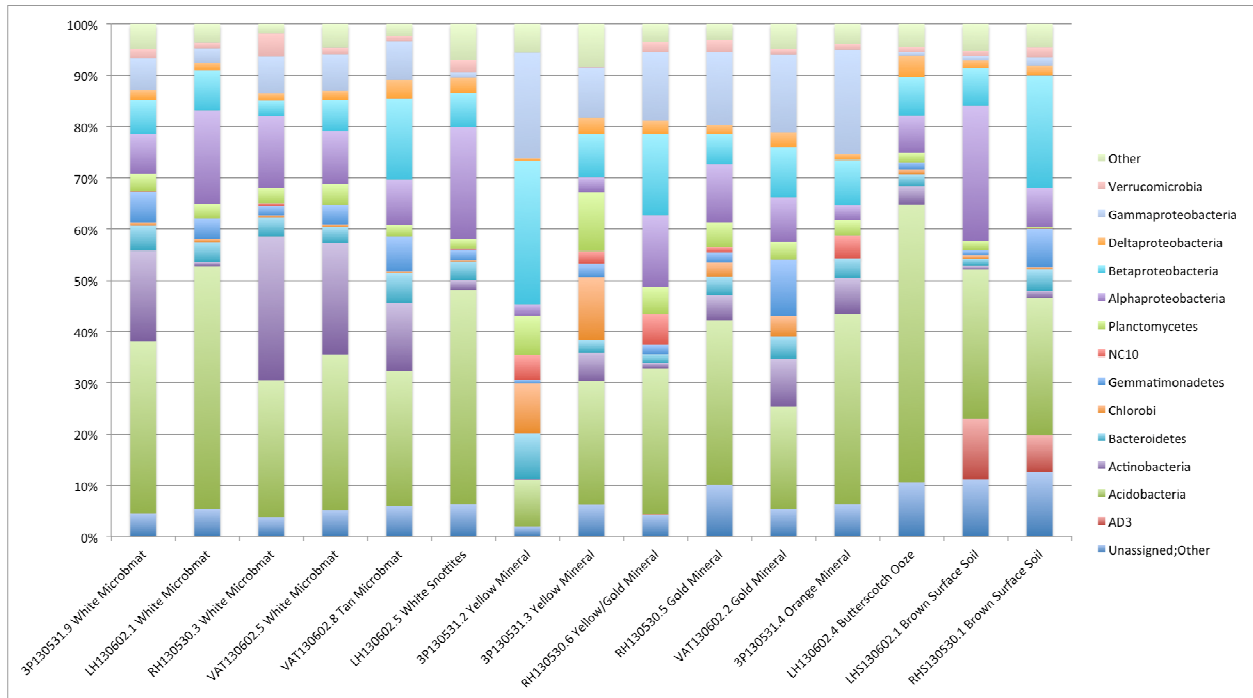


Figure 8: Bacterial phyla composition arranged by sample type, color, and cave.

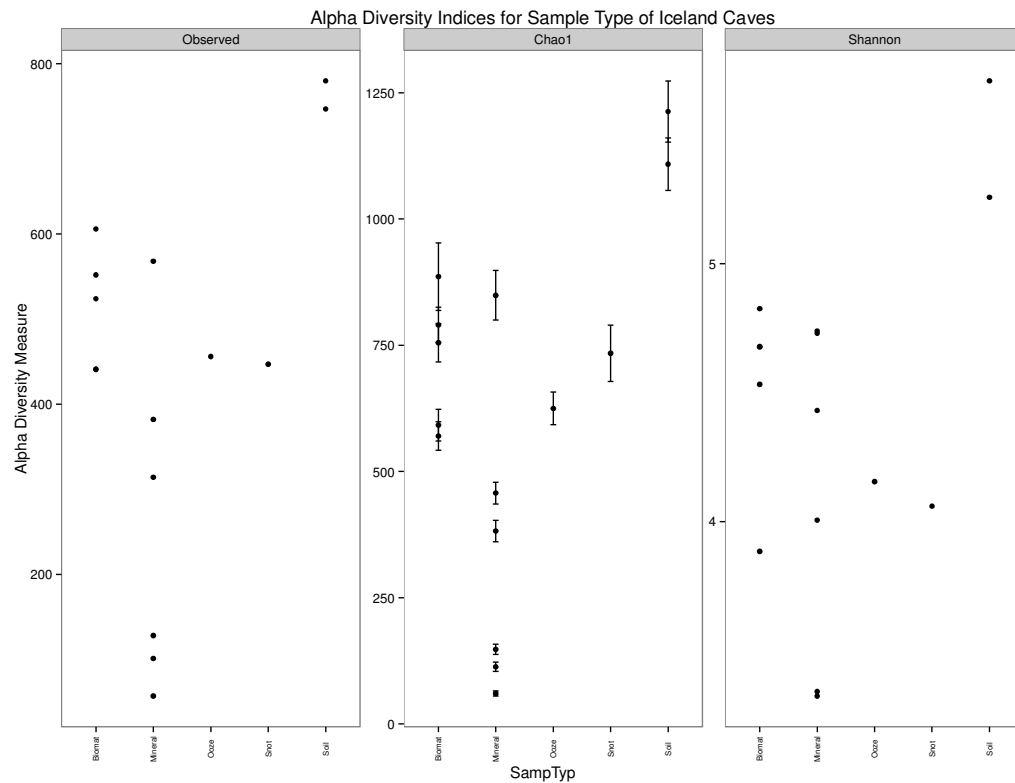


Figure 9: Alpha diversity by sample type: left to right on the x axis for each plot: biomat (microbial mat), mineral, ooze, snottite, surface soil.

Alpha diversity (the number of different operational taxonomic units (OTUs or “species”) present in any given sample) varied within and across sample types. Surface soils were the most diverse, while half of the mineral samples were the least diverse. Snottites and ooze were at the bottom end of the diversity observed in microbial mats (Fig. 9). The non-metric multidimensional scaling (NMDS) observed in Fig. 10 reveals that the two surface soil samples were substantially different from the cave samples (biomat,

mineral, ooze, and snot). The mineral and microbial mat (biomat) samples are mostly intermixed, revealing similarities in their makeup. Both the snottite and organic ooze samples were substantially different from the other cave samples.

NMDS plots by cave, color, surface versus cave, and by cave type were also done, but the most revealing was the substrate type, which appeared to be the most important factor so far studied in determining what controls diversity.

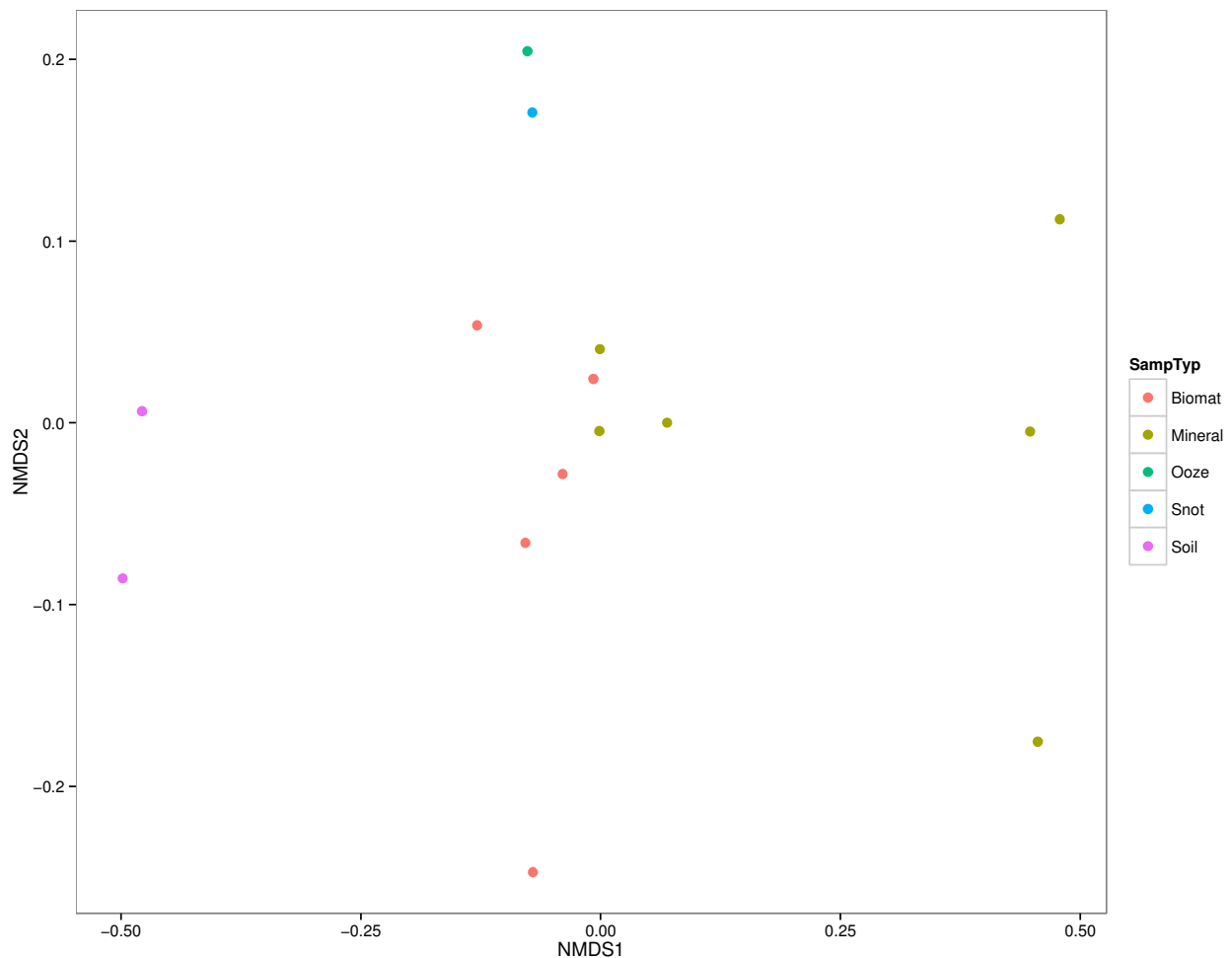


Figure 10: NMDS plot of diversity by sample type.

Conclusions

Samples of microbial mats, mineral deposits, a snottite, an organic ooze, and overlying surface soils were analyzed by SEM and DNA sequencing. SEM imaging revealed filamentous and coccoid morphologies, with biofilm present in some samples. DNA analysis demonstrated that the most abundant bacterial phyla present were the Actinobacteria, Acidobacteria and the Alpha-, Beta-, and

Gammmaproteobacteria. Surface soils were the most diverse, followed by microbial mat samples. The mineral samples varied from not very diverse to some being comparable to some of the microbial mats. Organic ooze and snottites were moderately diverse. Icelandic lava caves offer a new and exciting frontier for investigating microbial diversity in lava caves.

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