Exploring Microbes at Wind Cave as an Analog for Exobiological Environments off Earth

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Exploring Microbes at Wind Cave as an Analog for Exobiological Environments off Earth

Abby K. Sliwinski, Emma W. Pellegrino, Nicole Geerdes, and Marek K. Sliwinski

Abstract
Subterranean environments on Earth serve as an analog for the study of microbes on other planets. This has become an active area of research with the discovery of exoplanets. To learn about the microbial species living in Wind Cave, we are comparing methods to sample environmental DNA because most microbes cannot be cultivated using standard laboratory methods. We are then probing the environmental DNA with broad primers that are designed to amplify most life and narrow primers such as those specific to the domain Archaea. Of the methods compared, the Qiagen DNeasy PowerBiofilm kit produced the purest template as measured by its ability to be PCR amplified. The next steps are to optimize the DNA testing reactions to limit mispriming. In the future, these methods will be used to determine the identity, quantity, and spatial distribution of microbes in Wind Cave.

Background
As the field of astrobiology expands, renewed focus is being applied to the extreme environments on our own planet as an analog for life off Earth. Researchers in Hawaii studying lava caves and geothermal vents found that the microbial ecosystems were surprisingly more complex than expected (Prescott et al., 2022). These environments could mirror ecosystems on other planets such as ancient Mars. Another group of researchers have determined that ancient Mars could have supported life by producing hydrogen gas through radiolysis of water (Tarnas et al., 2018). Hydrogen gas is also used as an energy source by subterranean microbes on Earth. As a final example, a group at Stanford discovered that geological activity driven by the movement of tectonic plates creates change in subsurface microbial ecosystems (Zhang et al., 2022). They believe that this may be meaningful beyond Earth because tectonic shifts on other planetary bodies could produce the right environment for life. Our team is studying the microbial ecosystem at Wind Cave National Park in South Dakota to determine how it differs from the above ground community. These results may help in the search for life off planet.

Methods

Cave samples were collected from three areas of Wind Cave. The uppermost area of the cave was sampled at the Rat Scat Room (RSR). Two samples were collected from the lowest regions of the cave. The first was What the Hell Lake (WTH). The furthest sample was from Calcite Lake (CL). The images above show each sampling area.

Figure 1: Using E. coli to test DNA precipitation methods

DNA template
DNA Testing with Universal Primers

Of the three DNA extraction protocols tested here, the best was sequential extraction with SDS followed by EtOH. All three protocols produced visible DNA on the gel, as shown in the gel on the left. The sequential extraction had less deleterious contamination than the other methods (data not shown). All template DNA was sufficient purity to amplify in a PCR reaction using universal primers (gel on the right).

Results

Conclusions
Environmental DNA extracted with the Qiagen PowerBiofilm kit amplified better than DNA precipitated sequentially with ethanol and PEG. In the future, the sequential protocol could be optimized for samples with low numbers of microbes.

Cave samples were amplified by PCR, producing bands of the expected size. In the future, PCR needs to be optimized to reduce mispriming.

Citations

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The diagram above outlines the sequential DNA precipitation protocol. For the DNeasy PowerBiofilm kit (Qiagen catalog number 24000-50) the manufacturer’s protocol was followed except the final step was changed to two sequential 5 µL elutions. For gel electrophoresis, 0.7% agarose and Promega molecular weight marker 1kb (catalog number G5711) was used. To visualize DNA, loading dye included the Sybr green stain, GelGreen Nucleic Acid Stain 10.00x (catalog number GMD-500). PCR using 1 µL of extracted DNA was performed using OneTaq (catalog number M0482S) and with Phusion (catalog number F530S) for kit DNA. Cave sample images are shown on the right. 250 mg of each sample was used for DNA extraction.