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Biology

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 narrower 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 Hawai'i 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

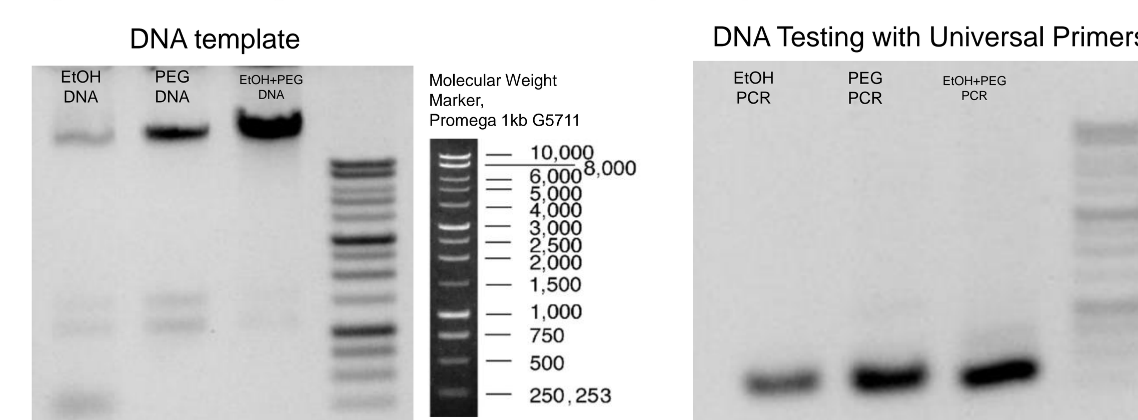
Cave samples were collected from three areas of Wind Cave. The uppermost area of the cave was sampled at the Rat Scat Room (RSR). Two samples were collected from the lowest regions of the cave. The first was What the Hell Lake (WTH). The furthest sample was from Calcite Lake (CL). The images above show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

Prepare samples		Resuspension step			Lysis step			First Precipitation			Second Precipitation			Resuspension
	pre-lysis rinse	Buffer	Chelator	Osmolarity	Detergent	enzyme	Incubation		carrier	spin	Add:	carrier	spin	
250 mg sample	TEN buffer	100 mM Tris pH 8	5 mM EDTA	200 mM NaCl	0.2% SDS	50 ug/mL ProK	60°C 15 min.	2x volume 95% EtOH	20 ug/mL LPA	20k	0.5x volume 30% PEG : 1.6 M NaCl	20 ug/mL LPA	20k	15 uL 10 mM Tris pH8

The diagram above outlines the sequential DNA precipitation protocol. For the DNeasy PowerBiofilm kit (Qiagen catalog number 24000-50) the manufacture's protocol was followed except the final step was changed to two sequential 50 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 10,000x (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.

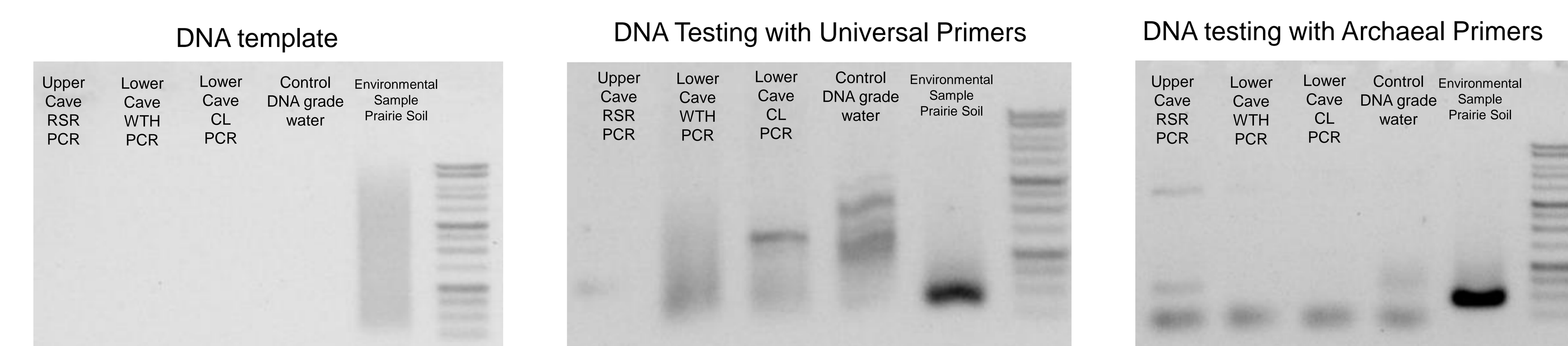
Results

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



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

Figure 2. Testing the sequential precipitation protocol to extract DNA from cave samples

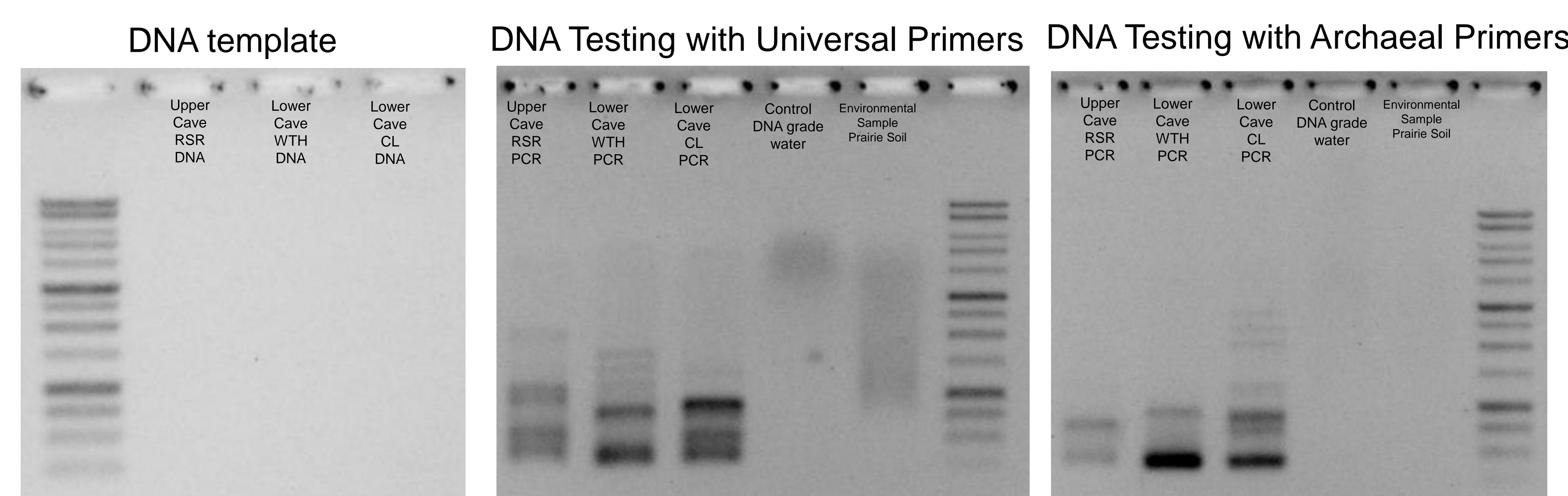


DNA was extracted from 250 mg of each sample. DNA was not detected for the cave samples by this method but may be below the limit of detection. As a positive control, prairie soil, which is know to contain a large number of microbes, was used.

DNA testing was conducted with a universal primer set that is able to amplify most known Bacteria and Archaea. The cave samples produced poor PCR results and lacked the expected band that is visible in the prairie soil sample.

DNA testing was conducted with a primer set for the domain Archaea. Mispriming produced a primer-dimer in the cave samples. The upper cave sample and the negative control both had faint bands of the expected size.

Figure 3. Testing the Qiagen Dneasy PowerBiofilm kit to extract DNA from cave samples



Cave samples extracted with the Qiagen Dneasy PowerBiofilm kit did not yield DNA bands visible on the gel. This is expected for samples with low numbers of microbes. DNA was below the limit of detection.

DNA testing using the universal primer set produced bands of the expected size in cave samples (between 250 – 500 bp on the size standard). PCR will need to be optimized to reduce mispriming and to improve amplification of the control prairie soil.

DNA testing using the archaeal primer set also produced bands of the expected size in cave samples (between 250 – 500 bp on the size standard). PCR will also need to be optimized to reduce mispriming and to improve amplification of the control.

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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