Species-Specific Quantification of Endospores in a Deep Subsurface Aquifer

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Introduction

It is estimated that between 12 and 20 percent of Earth's biomass is microbial and in the terrestrial subsurface (Magnabosco et al. 2018). A nearly universal trend in this ecosystem is the prevalence of bacteria from the phylum Firmicutes. Many members of this phylum form protective structures known as endospores. When a spore-former senses nutrient limitation or other environmental stress, it can undergo the process of sporulation, which involves the cessation of metabolism and formation of a protective spore coat. The ability to become dormant until more favorable conditions arise is particularly useful in extreme environments, such as the subsurface, allowing an individual spore to remain viable for potentially millions of years. Previous studies on abundance of spores in the subsurface focus on marine sediments. In the continental subsurface waters the abundance of spores is largely unknown. In this work we aim to determine the **species-specific concentration of spores relative to vegetative cells in the fractured rock aquifer Inyo-BLM 1.** The abundance and identity of spores compared to vegetative cells has interesting a variety of interesting implications **(Figure 1**).

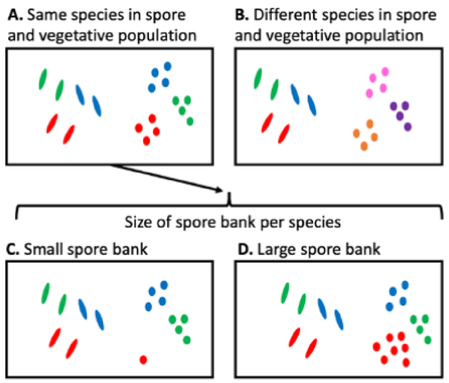
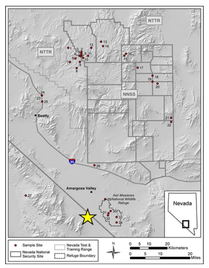


Fig 1. Visual representation of potential outcomes. For the purpose of this figure vegetative cells are represented by rods and spores are represented with circles. **A)** If the spore and vegetative populations are composed of the same taxa. **B)** If these populations are composed of different taxa. **C)** If A is true and a species has a small spore bank relative to the vegetative population. **D)** If A is true and a species has a large spore bank relative to the vegetative population.

Site Description

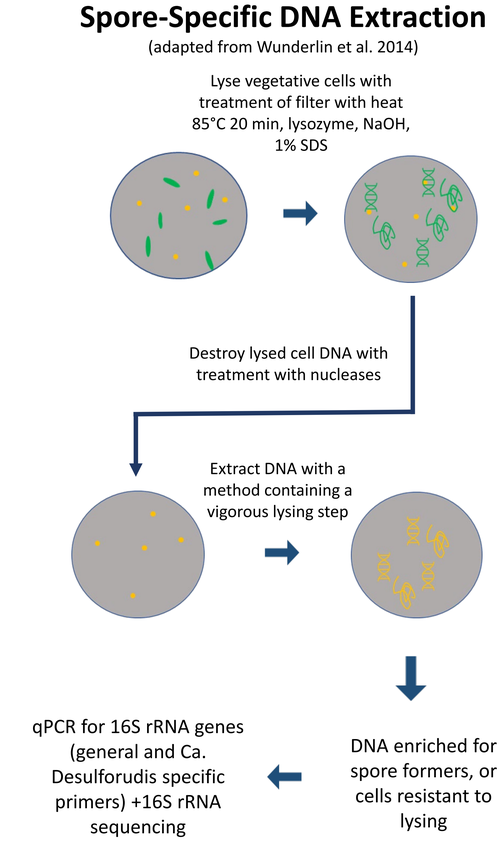


Inyo-BLM-1 is a water-filled borehole located at 36.4004°N, -116.4692°W in the discharge zone of the Death Valley Regional Flow System (DVRFS), a fractured rock aquifer beneath portions of western NV and eastern CA The hole is continuously cased to 750 m through lake sediments, volcanic tuff, and alluvium, ultimately accessing a hydrologically productive zone in Hidden Valley Dolomite.

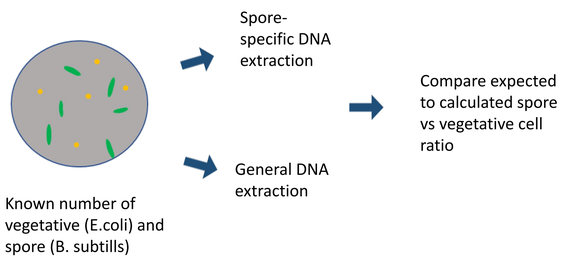
Methods

Spore-Specific DNA Extraction

(adapted from Wunderlin et al. 2014)



Benchmarking (for efficiency and specificity)



Results

16S rRNA Gene Amplicon Data for Non-Specific DNA Extraction from BLM-1

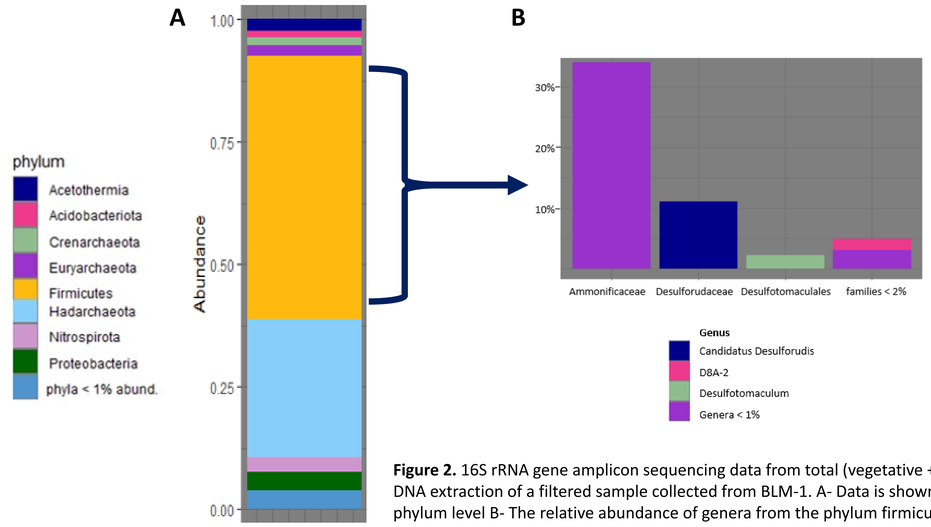


Figure 2. 16S rRNA gene amplicon sequencing data from total (vegetative + spore) DNA extraction of a filtered sample collected from BLM-1. A- Data is shown at the phylum level B- The relative abundance of genera from the phylum firmicutes.

Benchmarking Spore-Specific Extraction with *B. subtilis* Spores and *E. coli* Vegetative Cells

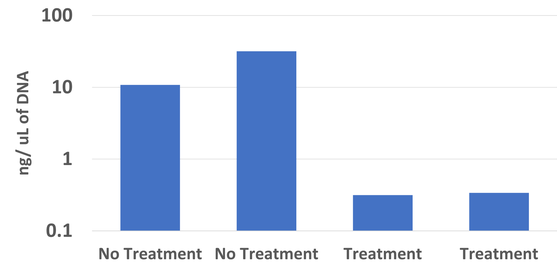


Figure 3. Results of spore-specific extraction on a mixture of approximately 90% E.coli cells (vegetative) and 10% purified B. subtilis spores and non-extraction control.

- The relative abundance of the phylum Firmicutes (some of which form bacterial endospores AKA spores) in the vegetative + spore form in BLM-1 is 54% suggesting this is a good environment to look for spores **(Figure 2A).**
- Known spore-forming taxa such as *Candidatus* Desulforudis is at a relative abundance of 11 % and a suspected spore-forming taxa "unknown genera in the Ammonificaceae family" is at a relative abundance of 34% **(Figure2B).**
- Reduction in recovered DNA from the spore-specific extraction indicated this treatment effectively lysed and destroyed the DNA of the vegetative cells. Loss of spore DNA was within an order of magnitude **(Figure 3)**

Future Directions

- **Complete spore-specific DNA extractions, quantify 16S rRNA gene copies in the spore fraction, and sequence 16S rRNA gene amplicon library to determine the identity and quantity of spores in this environment.**

Acknowledgements

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References

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