Modified techniques to extract and amplify soil DNA for studying ciliate biodiversity

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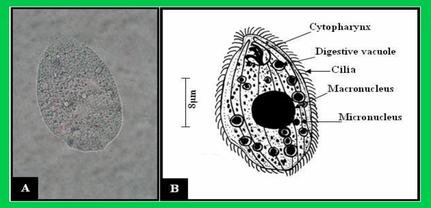
Introduction

- Ciliates are a relatively under-researched protozoan.
- About 8,000 species have been discovered.
- Ciliates play many important roles in soil ecosystems such as eating bacteria. (1)
- Discovering the biodiversity of soil ciliates to understand soil health was the main focus.



Example of a ciliate and its morphological features. (2)

Methods

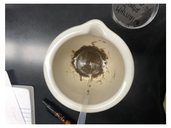


Figure 1: Metagenomic soil DNA extraction

- Soil Collection
- Finding and culturing ciliates - protozoa
- Metagenomic soil DNA extraction
  - glass beads
  - charcoal
- DNA purification
- Gel electrophoresis
- Spectrophotometry
- PCR reaction

Figure 2: Brazilian Bluewood Tree

Methods

- Soil was collected from the tree in-between Tidwell and Waco Hall (Figure 2).
- Glass beads were added to breakdown the soil, based on a silica bead extraction protocol (Figure 1).
- Charcoal powder was added to remove impurities from the soil (Figure 1).
- Soil purification was done by using a vacuum filtration manifold and doing a total of three washes of the Wizard Genomic DNA purification Kit (Promega)
- PCR was performed using 18S V4 primers (3)

Results

- Soil sample was mainly sand.
- Gel electrophoresis: DNA was in the sample (figure 3)
- Spectrophotometry: ng/ul = 169.6, A260/A280 = 1.43, A260/A230 = 0.64.
- PCR reaction: Negative, possibly due to impurities in the soil.

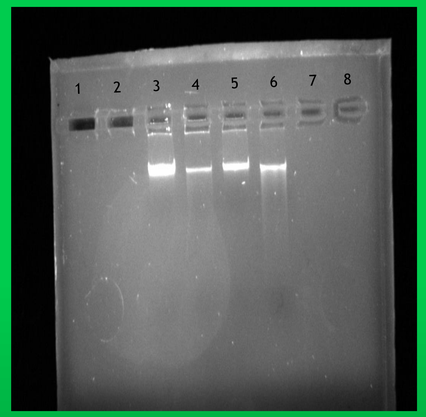


Figure 3. Gel electrophoresis.
Lane 1 and 2 are empty
Lane 3: 10ul of DNA sample
Lane 4: DNA mass 125ng
Lane 5: DNA mass 63ng
Lane 6: DNA mass 250ng
Lane 7 and 8 are empty

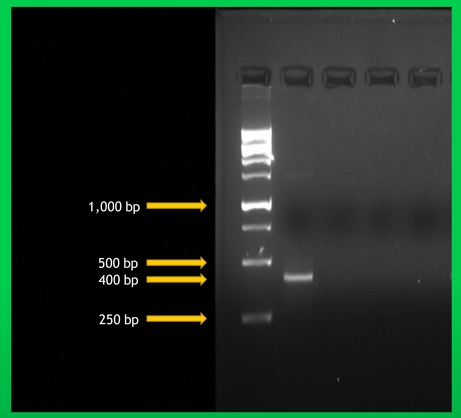


Figure 4. Gel Electrophoresis of PCR products
Lane 1: 1kb ladder
Lane 2: Sample HHL
Lane 3: Negative control
Lane 4: Sample MT
Lane 5: Negative control

Discussion

- Although there was a negative PCR result, other samples came back positive which means the DNA collection method worked.
- Negative PCR result may have been because of impurities in the soil such as human substances
- Future researchers should consider taking more time to purify the DNA and do several dilutions.

Conclusion

- Impurities inhibit the PCR reaction.
- PCR worked on 5 out of 8 samples, meaning that the DNA extraction technique worked, and future researchers can use it.
- Positive PCR results will be sent out for sequencing and that will give us the data to determine the diversity of ciliates in our soil.

References

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3. Stoeck, Thorsten, David Bass, Markus Nebel, Richard Christen, Meredith D. M. Jones, Hans-Werner Breiner, and Thomas A. Richards. "Multiple Marker Parallel Tag Environmental DNA Sequencing Reveals a Highly Complex Eukaryotic Community in Marine Anoxic Water." *Molecular Ecology*19 (2010): 21-31.