DNA in Ancient Plant Microfossils?

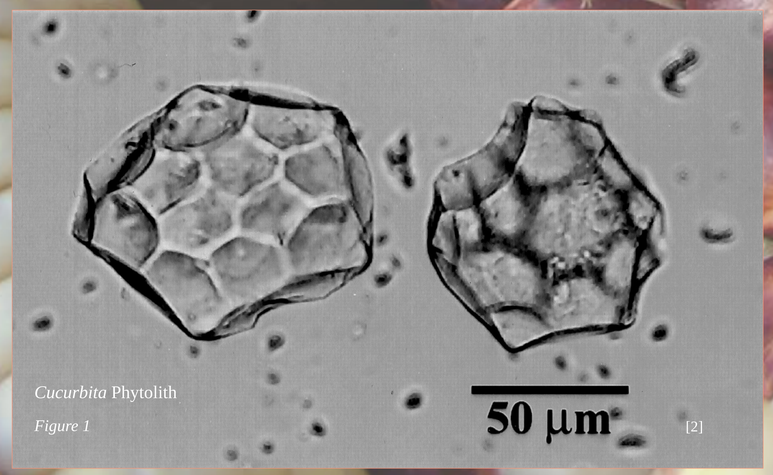
Andrea N. Marroquin[1,3], S. Yoshi Maezumi[2], Logan Kistler[3]
[1]Northern Kentucky University, [2]University of Exeter,[3]Smithsonian Institution of Natural History

Introduction

Just like remains of clay pots, wooden toys, and deserted homes, crop remains from ancient civilizations can tell a lot about the people who lived there. Crop remains inform archeologists how those plants have changed over the time they have been domesticated. A major problem, however, is that most domesticated plant remains do not preserve well over time. There is much information on plant domestication in North America because plants like corn, Zea mays, and squash, Cucurbita, have preserved well enough in some contexts to recover DNA from plant tissues, even after several thousand years. Environments like the rain forest and other harsh climates do not allow plants used in those areas to preserve well over archaeological timespans.

Phytoliths (Figure 1), however, are silica microparticles found in many plant tissues, and they are very durable even in conditions where tissues degrade rapidly. When the phytoliths were forming within the plants, it is unknown whether DNA can be integrated in the amorphous “glass” of the phytoliths. If so, this raises the possibility that DNA within plants can be recovered even from harsh preservational environments, allowing the use of archaeogenomic methods to probe new regions and even earlier deposits, for example the onset of domestication in lowland Mesoamerica.

A protocol was introduced by Dr. Daniela Paunescu at Functional Materials Lab in Switzerland to test the idea of DNA within phytoliths with the use of synthetic DNA and silica, in the paper. *Reversible DNA encapsulation in Silica to produce ROS-resistant and heat resistant synthetic DNA ‘fossils’* [1]. We tested this protocol with naturally formed phytoliths to see if DNA can successfully be recovered as a first step toward ancient DNA research using phytoliths.



Cucurbita Phytolith

Figure 1 [2]

Methods

Phytoliths were extracted from modern *Zea mays* and *Cucurbita moschata* tissues for DNA experimentation using established protocols. We then tested for DNA presence in phytoliths as follows:

- Primers were selected from a gene selection of *Zea mays* and *Cucurbita* that would show genes as specific to that species as much as possible
- Comparative DNA was extracted from the silk of sweetcorn and flesh of zucchini, purchased from Safeway grocery store, to optimize primer sets.
- Polymerase chain reaction (PCR) conditions were optimized for two primer sets in each species.
- 300 µL of a dilute fluoride-based etching solution was used to dissolve phytoliths to saturation.
- DNA purification was completed with Qiagen MinElute kits according to the manufacturer’s protocol, and 50 µL PCR reactions with positive and negative controls were used to test for DNA presence.

Discussion

The expected band was not recovered during PCR, and we hypothesized that more phytoliths were necessary to yield amplifiable DNA being dissolved so the amount of etching solution was increased to 1mL. For both the *Zea mays* and *Cucurbita* phytoliths, there was no positive sign of the correct band in the gel. To test whether etching solution may be destroying the DNA or impeding recovery, we repeated phytolith extraction with high-quality tissue DNA spiked in as a positive control. We also used water in place of etching solution in a positive control. Following extraction, we amplified the results alongside a positive DNA control and a negative water control.

Figure 2 shows the results. We found that the template DNA strand made it through only the positive control containing only DNA and water, no etching solution, indicating that the etching solution either damages DNA or impedes recovery. We performed another test for whether DNA can survive the etching solution at different concentrations, seen in Figure 3. The DNA made it through the dilution of 150uL water and 50uL of etching solution, but rapidly declined at higher concentrations.

Figure 2

Conclusion

Our results have been inconclusive as to DNA preservation in plant silica bodies, but we have taken steps to identify and remedy methodological obstacles. The original protocol was developed for very thin silica structures requiring little etching solution, and the extra etching solution, though diluted, might be damaging the DNA isolation columns. The question of whether or not there is DNA present in the phytoliths is unanswered.

Figure 3

References

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