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# Development of Molecular Tools for Identification of Prairie Terrestrial and Wetland Algae

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## ABSTRACT

Algae are a diverse group of photosynthetic protists. Green algae are typically unicellular, though some may occur as multicellular colonies, and they are found across a wide range of habitats, including marine, freshwater, and terrestrial environments. As photosynthetic producers and sometimes symbionts, algae can occupy important roles in an ecosystem; in other circumstances they can have negative impacts on environmental quality. Estimations of the number of green algal species range from eight thousand to over fifteen thousand. The identification of individual algal species can be problematic due to their small size and typically simple cell anatomy. For this reason, molecular tools may be employed to distinguish between algae. We have examined the utility of inter-simple sequence repeat (ISSR) analysis to generate a "bar code" molecular phenotype for the identification of different green algae. Environmental samples were collected from UNO's Glacier Creek Preserve and from other local sites. Algae from these sites were cultured and isolated. Genomic DNA from these algae was used as the template for PCR amplification, both with and without initial purification from the algae. Amplification of the high copy number ribosomal internal transcribed sequence was possible without initial DNA purification, yielding characteristic size products for different algae; this method did not reliably work for amplification using ISSR primers. Isolated algal DNA was tested with >50 ISSR primers to identify primers that could generate distinct amplification patterns that might be useful for rapid identification of algal species.

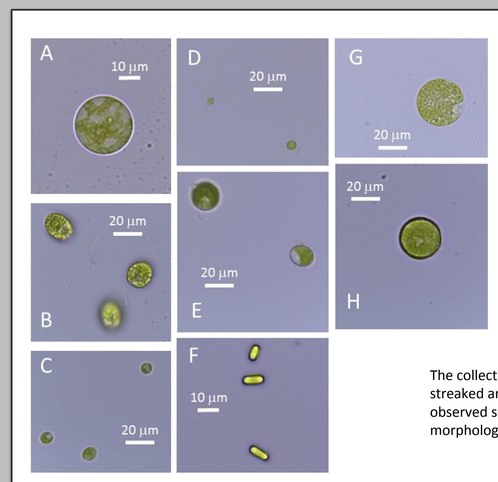



**Figure 2. Isolated algae from Glacier Creek Preserve**, viewed at 1000x magnification.
A. Culture 1B.
B. Culture 2A.
C. Culture 2B.
D. Culture 3B.
E. Culture 4A.
F. Culture 5B.
G. Culture 6A.
H. Culture 7A.

The collected Algae were streaked and isolated. We observed several distinct morphologies.

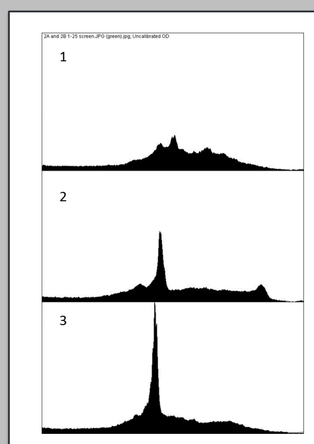



**Figure 5. Comparison of gel image density plots from different primers.** To make the interpretation of amplification patterns less subjective, gel lanes image densities were plotted using ImageJ. The left side of the image corresponds to the top of the gel lane. These patterns were generated using genomic DNA from Culture 2B (see Figure 4). 1, primer 12; 2, primer 13; 3, primer 14. Distinct patterns generated from different algae may be compared by aligning plots from the same primer amplifying from different genomic templates.


**Figure 1. Collection sites at Glacier Creek Preserve.** Water samples collected from moving and standing water on May 04, 2016. Numbers show collection sites. Numbers correspond to sites identified in Table I. Algae were cultured from water samples using a mineral nutrient medium. Image: Google Maps.

**Table II.** ISSR primers used in this study.

| No. | Seq. | No. | Seq. | No. | Seq. | No. | Seq. | No. | Seq. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | (AC)8C | 6 | (AG)8G | 11 | (ACC)6T | 16 | (AGT)6C | 21 | (CA)8A |
| 2 | (AC)8G | 7 | (AG)8T | 12 | (AGC)6 | 17 | (AGC)6T | 22 | (CA)8G |
| 3 | (AC)8T | 8 | (ACC)6 | 13 | (AGC)6C | 18 | (AGT)6G | 23 | (CA)8T |
| 4 | (AG)8 | 9 | (ACC)6C | 14 | (AGC)6G | 19 | (AGT)6T | 24 | (CT)8 |
| 5 | (AG)8C | 10 | (ACC)6G | 15 | (AGT)6 | 20 | (CA)8 | 25 | (CT)8A |

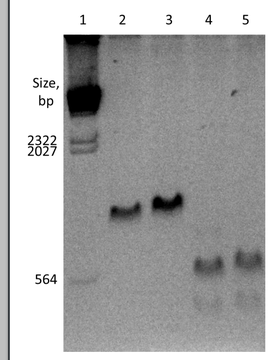


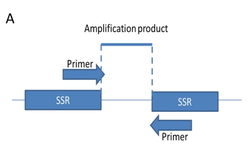

**Figure 6. Restriction endonuclease analysis of ITS amplification product**. The ribosomal ITS amplification product from Culture 2B was subject to restriction endonuclease digestion to determine if the resulting fragment pattern could be used as a tool for distinguishing algal species.

Lane 1: Marker
Lane 2: No enzyme
Lane 3: *Eco*RI
Lane 4: *Eco*RV
Lane 5: *Eco*RI + *Eco*RV

The generation of a distinct cutting pattern suggests that restriction analysis of amplified ITS fragments may be a useful approach to distinguishing different algae.

**Table I.** Environmental Conditions at Glacier Creek Preserve sampling sites.

| Sites | Oxygen (mg/L) | Temperature (C) |
|---|---|---|
| 1. Wet Land | 5.7 | 18 |
| 2. Glacier Creek Upstream in Woods | 6.4 | 16 |
| 3. South Pond | 5.0 | 14 |
| 4. Standing Water | 5.3 | 20 |
| 5. Flowing water mid course | 9.1 | 16 |
| 6. Standing water mid course | 4.1 | 21 |
| 7. Downstream of Confluence | 10.6 | 15 |



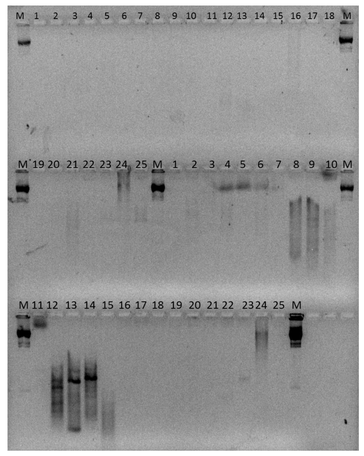




**Figure 4. ISSR strategy and primer screening. A.** Inter-simple sequence repeat (ISSR) analysis amplifies regions of a genome that occur between repetitive sequences distributed throughout the genome. The resulting banding pattern may be used as phenotype of the organism. **B.** Primer screening determines which patterns will generated using genomic DNA from cultures 2A and 2B as templates. Top and left side of middle row, Culture 2A amplified with ISSR primers 1-25. Left side of middle row and bottom row, Culture 2B amplified with ISSR primers 1-15. M = marker. Note distinct patterns generated from the two genomes. Amplification conditions were similar to those describe in Mostafa *et al.* (2011).

## Conclusions and Future Directions

Several morphologically distinct algae could be cultures from the Glacier Creek Prairie Preserve, suggesting a diverse community.

Amplification of the ribosomal Internal Transcribed Spacer from isolated algal genomic DNA yielded differently sized products, indicating that these isolates are genetically distinct from each other.

Application if ISSR primers to genomic templates also generated distinct amplification patterns that might be useful in identifying particular algal species when encountered.

Restriction endonuclease cutting of amplified ribosomal ITS products also generated distinct patterns that might provide an additional molecular phenotype for distinguishing between different algae.

**References**

Mostafa, N., Omar, H., Tan, S. G., & Napis, S. (2011). Studies on the genetic variation of the green unicellular alga Haematococcus pluvialis (Chlorophyceae) obtained from different geographical locations using ISSR and RAPD molecular marker. *Molecules*, *16*(3), 2599-2608.



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