

matK Gene Sequencing Suggests High Genetic Diversity in Shaw's Agave and Enzyme Activity Assays Reveal Diverse Microbial Activity Within Pt. Loma Ecological Preserve

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Summary

- *Null Hypothesis*: The Shaw's agave in Coastal Southern California and Baja California are genetically and microbially diverse.
- Phylogenetic trees were generated using DNA barcode region *matK*, and using Shaw's Agave samples from four sites.
- Metabolic enzyme profiling assays were used to determine carbon source utilization by soil microbes.
- There is genetic diversity within the population of Shaw's agave at Point Loma, and differences in carbon source utilization by plant soil microbes.

Abstract

National Park Services is concerned that the native Shaw's agave will become extinct in the coming decades and have launched efforts to study and protect the plant. Stemming from concerns that the plant may be clonally reproduced, genetic diversity of the Shaw's Agave were examined using samples from four different regions: Point Loma (Cabrillo National Monument, Navy Base), Border Fields, Rosarito, and Arroyo Hondo. Phylogenetic trees were generated using the DNA barcode region *matK*. Microbial enzyme assays were additionally utilized to identify and quantify carbon source utilization by soil microbes. Our results indicate that there is genetic variation within the population of Shaw's agave at Point Loma and diversity in carbon sources utilized by microbes.

Introduction

Background

- Shaw's agave primarily grows along the coast of California (U.S) and Baja California (Mexico)¹. (Fig. 2)
- A standard barcoding gene for land plants, *matK*, was used to determine the genetic variation within the Shaw's Agave population in Pt. Loma.
- *matK*² is a chloroplast gene that codes for maturase K, an enzyme that is involved in intron splicing.
- Microbial communities in the soils of these Agave plants were analyzed to assess the health status of the plant.



Figure 1. Cluster of flowering *Agave shawii* rosettes from Cabrillo National Monument. The above image was taken near the tidepools of the park, adjacent to an eroding cliff.

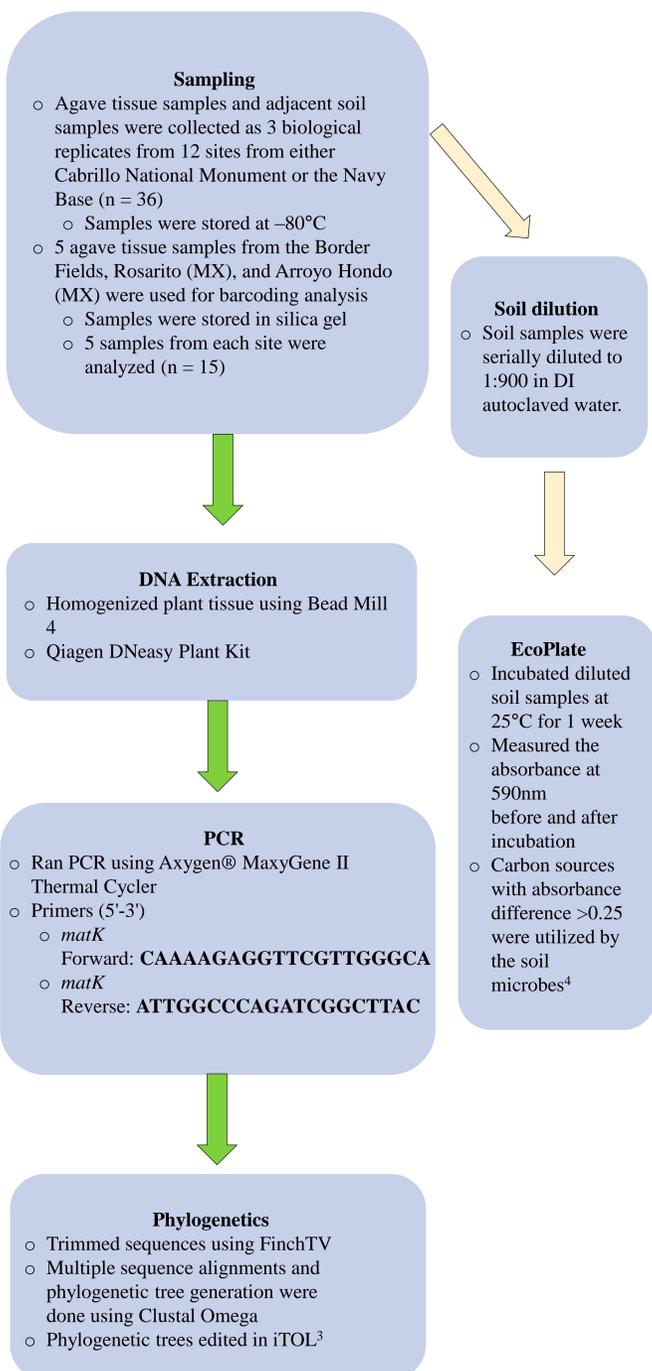


Figure 2. Range of *Agave shawii* growth. The red highlighted area in the above map mark the known bounds of where Shaw's agave typically grown. From North to South, this area includes: 1. Pt. Loma, 2. Border Fields, 3. Rosarito, MX, and 4. Arroyo Hondo, MX.

Significance

Shaw's agave is an endangered, that is currently not protected by any federal agency. With the plant's long reproductive cycle, low numbers of viable seeds, and habitat loss, the population of this species may continue to decline. Understanding the genetic and microbial diversity of this agave in Point Loma will aid National Park Services (NPS) in restorative efforts for the Shaw's agave.

Methodology



Results

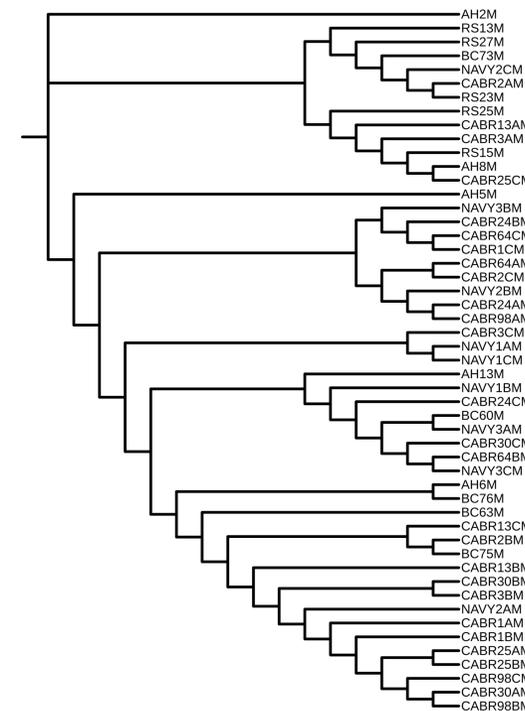


Figure 4. Unrooted phylogenetic tree of Shaw's Agave generated in iTOL using *matK* sequences. Sample names consist of geographic source (e.g. 'CABR' or 'NAVY'), plant identifier, and biological replicate (e.g. A/B/C). BC: Border Fields, RS: Rosarito, AH: Arroyo Hondo. The suffix 'M' in the above figure refers to the gene *matK*. Phylogenetic trees were generated in Clustal Omega and further edited in the Interactive Tree of Life (iTOL).

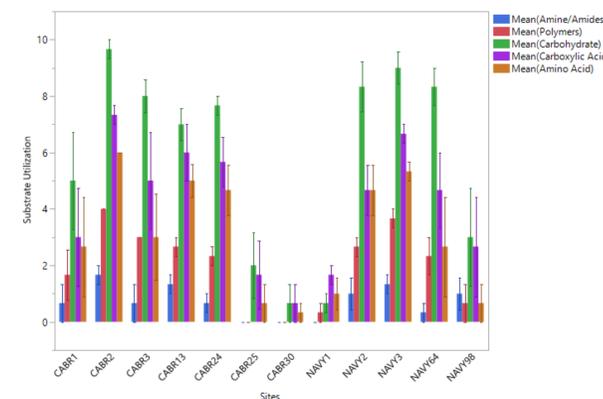


Figure 5. Mean substrate utilization by sites in Cabrillo and Navy Base. The mean number of substrates used per carbon source (e.g. 'Amines/Amides', 'Polymers', etc.) per site is displayed above with each error bar constructed using one standard error from the mean. Average well color development from Biolog EcoPlates were calculated according to Gryta et al.⁵ Substrate utilization is defined as a positive test for the well; the average well color development must be > 0.25 to be considered a positive test⁴. Substrate utilization or "richness" represents the number of different sources used by the microbes.

Conclusion

The barcoding region *matK* showed genetic diversity within Point Loma (Fig. 4). The interleaved samples from Border Fields, Rosarito, Arroyo Hondo between Point Loma samples in both phylogenetic trees suggests that the Shaw's agave in Point Loma are not all clonally propagated. Analysis of the soil microbe substrate utilization also showed dissimilarities in microbial carbon source utilization, suggesting functional diversity (Fig. 5). Sites such as CABR 25, CABR 30, and NAVY 1 use few carbon sources while nearby sites such as CABR 24, and NAVY 2 utilize much more.

Limitations:

- Barcoding gene regions for phylogenetic analysis may have not revealed complete underlying variation. Utility of more expansive hypervariable loci may have been more appropriate for comprehensive phylogenetic analysis.
- Lack of a conclusive morphological phenotype diversity among Agave from geographically distinct utilized sites limits site-specific gene-environment interaction analysis and evidence of speciation.
- Soil samples were only collected during single dry-season event. Variation caused by environmental fluctuation was not accounted for.

Future Directions

In this study, the genetic diversity of the Shaw's agave population and the functional diversity of adjacent microbial communities was determined. In order to draw further conclusions from the EcoPlate data, soil microbes from our soil samples need to be quantified and identified.

References

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