Barcoding genes *rbcL* and *matK* reveal Shaw's Agave genetic diversity while Biolog EcoPlates quantify variation in microbial substrate utilization within Pt. Loma Cabrillo National Monument

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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 regions *rbcL* and *matK*, and 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

The Shaw's agave is an integral part of the environment that should be safeguarded to protect the delicate ecosystem. Genetic diversity of this 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 regions *rbcL* and *matK*. Microbial enzyme assays were 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.

Introduction

Background

- The habitat of Shaw's agave is limited to the coastlines of California and Mexico[1] (Fig. 2)
- DNA barcoding was used to identify the species and genetic diversity of Shaw's agave using the standard barcoding genes for land plants: *rbcL*[2] and *matK*[3].

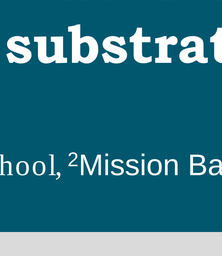

Figure 1. Cluster of three *Agave shawii shawii* rosettes from Cabrillo National Monument. The above image was taken near the tidepools of the park.

Significance

Shaw's agave is endangered, with only ~1,000 individuals left in California[1]. The declining population is due to transplantation, long reproductive cycles, and anthropogenic and natural habitat loss[1]. 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.

Figure 2. Range of *Agave shawii shawii* growth. The red highlighted area in the above map mark the known bounds of where Shaw's agave typically grow

Acknowledgements

We would like to thank and acknowledge Sula Vanderplank for collecting Shaw's agave samples throughout Baja California, and the National Park Services for generously funding this project.

Materials & Methods

Sampling
- Agave tissue samples and adjacent soil samples were collected as 3 biological replicates from 12 sites from either Cabrillo National Monument or the Navy Base (n = 36)
  - Samples were stored at –80°C
- 5 agave tissue samples from the Border Fields, Rosarito (MX), and Arroyo Hondo (MX) were used for barcoding analysis
  - Samples were stored in silica gel
  - 5 samples from each site were analyzed (n = 15)

DNA Extraction
- Homogenized plant tissue using liquid nitrogen and mortar/pestle
- Qiagen DNeasy Plant Kit

PCR
- Ran PCR using Axygen® MaxyGene II Thermal Cycler
- Primers (5'-3')
  - *rbcL* Forward: **CTGCGAATTCCCCCTGCTTA**
  - *rbcL* Reverse: **GATCGCGTCCCTCATTACGA**
  - *matK* Forward: **CAAAAGAGGTTCGTTGGGCA**
  - *matK* Reverse: **ATTGGCCCAGATCGGCTTAC**

Phylogenetics
- Trimmed sequences using FinchTV
- Multiple sequence alignments and phylogenetic tree generation were done using Clustal Omega
- Phylogenetic trees edited in iTOL[4]

Soil dilution
- Soil samples were serially diluted to 1:900 in DI autoclaved water.

EcoPlate
- Incubated diluted soil samples at 25°C for 1 week
- Measured the absorbance at 590nm before and after incubation
- Carbon sources with absorbance difference >0.25 were utilized by the soil microbes[5]

Results

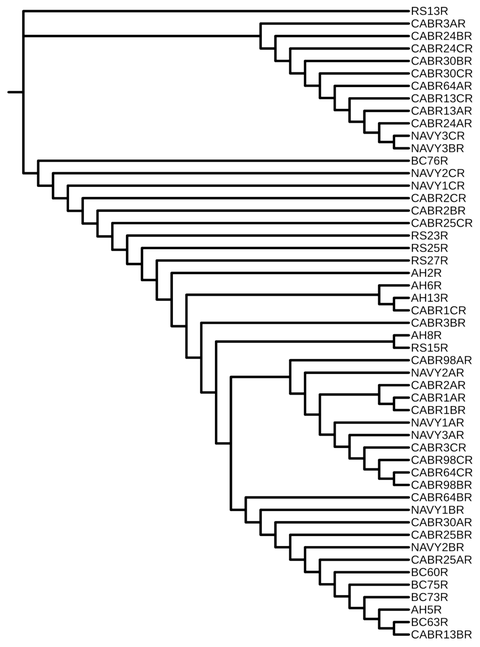

Figure 4. Unrooted phylogenetic tree of Shaw's Agave generated in iTOL using *rbcL* sequences. Sample names consist of geographic source (e.g. 'CABR' or 'NAVY'), plant identifier, and biological replicate (e.g. A/B/C). BC: Border Fields, RS: Rosarito, AH: Arroyo Hondo. The suffix 'R' in the above figure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

Results

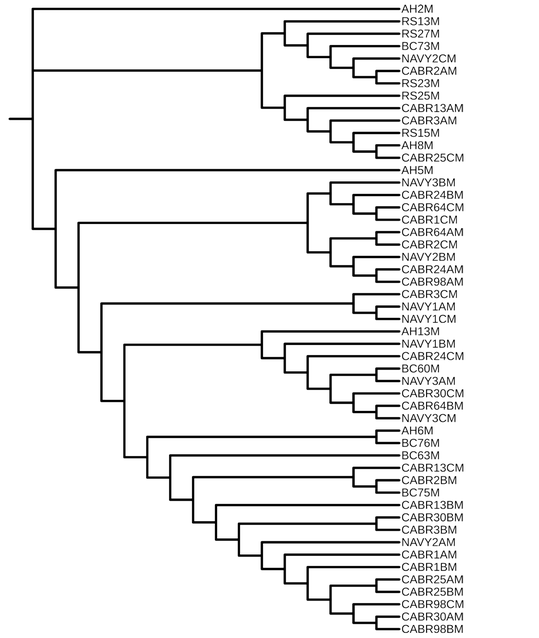

Figure 5. Unrooted phylogenetic tree of Shaw's Agave generated in iTOL using *matK* sequences. Sample names consist of geographic source (e.g. 'CABR' or 'NAVY'), plant identifier, and biological replicate (e.g. A/B/C). BC: Border Fields, RS: Rosarito, AH: Arroyo Hondo. The suffix 'M' in the above figure refers to the gene *matK*. Phylogenetic trees were generated in Clustal Omega and further edited in the Interactive Tree of Life (iTOL).

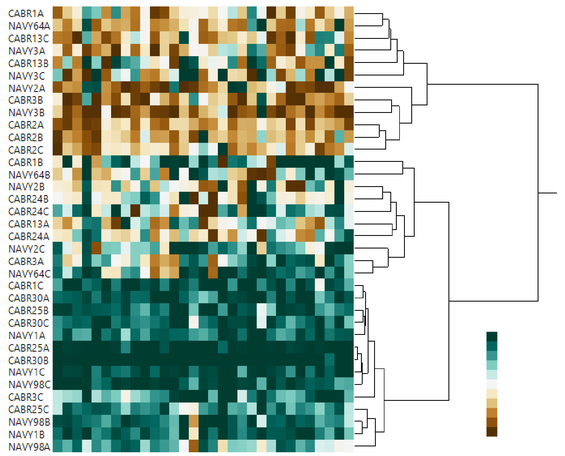

Figure 6. Hierarchical cluster of carbon source utilization by microbes from Pt. Loma soil samples. Samples are identified by location (e.g. 'CABR' or 'NAVY'), adjacent plant identifier (e.g. 1-98), and biological sample 'A/B/C'. From lowest to highest values, the heatmap ranges from blue to white to brown. Calculations were done according to Gryta et al [6]. Each row in the above heatmap represents a sample whilst each column represents a carbon source from the Biolog EcoPlate[6]. 590nm absorbance readings from the negative controls were subtracted from sample readings. Pre-incubation absorbance readings were then subtracted from post-incubation readings to determine well color development. Hierarchical clustering was generated in JMP Pro.

Results

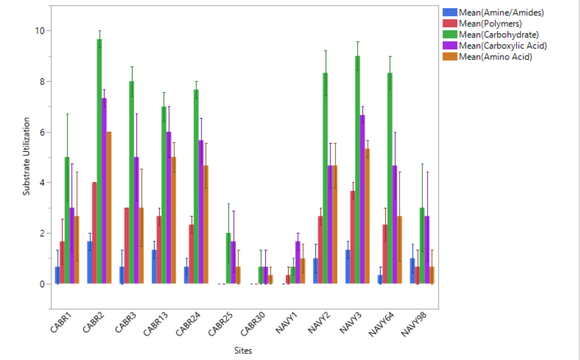

Figure 7. Mean substrate utilization by sites in Cabrillo and Navy Base. The mean number of substrates used per carbon source (e.g. 'Amines/Amides', 'Polymers', etc.) per site is displayed above with each error bar constructed using one standard error from the mean. Substrate utilization is defined as a positive test for the well; the average well color development must be > 0.25 to be considered a positive test [5]. Substrate utilization or "richness" represents the number of different sources used by the microbes.

Conclusion/ Discussion

The barcoding regions *rbcL* and *matK* show genetic diversity within Point Loma (Fig. 4, Fig. 5). 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 shows three different clusters of sites dissimilar in microbial carbon source utilization, suggesting functional diversity (Fig. 6).

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.
- For the experiments, technical replicates were not performed.

Future direction:

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.

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References