Mining the Agave Microbiome for adaptations to arid environments

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Introduction: A major challenge facing the biofuels industry is the identification of high-yield plant feedstocks that can be cultivated with minimal resource inputs without competing for land and water supplies with existing food crops. Recent research has demonstrated that the *Agave* plant, cultivated in Mexico and Southwestern United States for the production of fiber and alcohol, meets these criteria[1]. Agaves grow on non-arable rocky soils in regions characterized by prolonged drought and extreme temperatures, due in part to physiological adaptions that prevent excess water-loss in arid environments[2]. Plant-microbial symbioses can play a role in helping plants adapt to heat and drought stress, increasing the accessibility of soil nutrients, or compete with plant pathogens[3]. Whether agaves have similar beneficial microbe interactions in their native environment is unknown. We aim to provide a comprehensive characterization of the *Agave* microbiome, with the goal of identifying specific community members that may contribute to *Agave* biotic and abiotic stress tolerance.

Sampling Plan: We are investigating the microbial communities of both wild and cultivated *Agave* species. Agave specimens and associated soil samples were collected from three sites in Southern California and from four sites in Central Mexico. In California, all samples were collected from the wild species *Agave deserti*. In Mexico, samples of cultivated *Agave tequiliana* were collected from two Agave plantations, while the species *Agave salmiana* was collected from its native habitat. For comparison, a smaller number of samples were collected from two species of native cacti (*Myrtillocactus geometrizans* and *Opuntia robusta*).

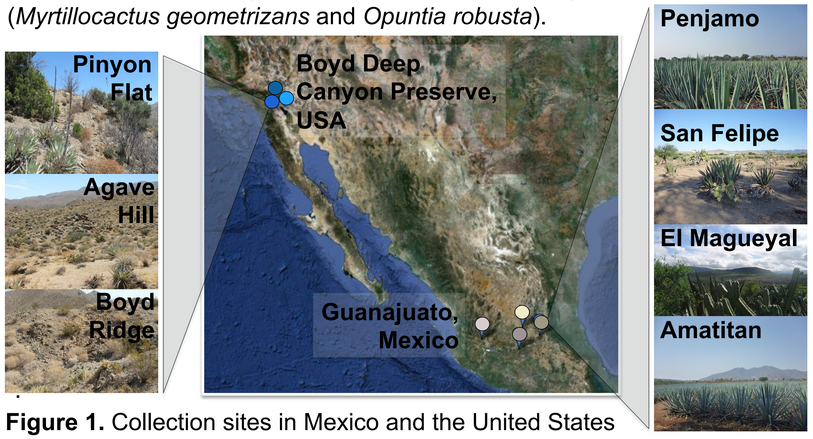

Figure 1. Collection sites in Mexico and the United States

Sample Types:

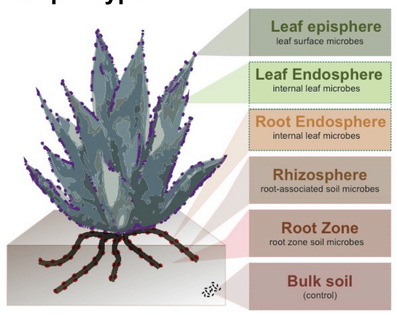

Figure 2. The six sample types collected from all plant specimens.

Sample Times:

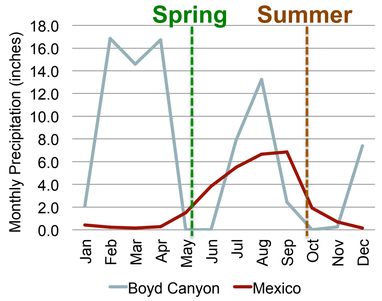

Figure 3. Two sample collection points, in late spring and summer.

For each Agave plant, samples were collected from the leaf episphere (leaf surface), rhizosphere (root surface) and leaf and root interiors (endospheres), as well as from surrounding soils (root zone and bulk soil). Additionally, sampling was repeated over two time points in 2012 (spring and summer), corresponding to the beginning and ends of the rainy season.

iTag Analysis Pipeline: A 16S iTag survey of all samples is being used to select specific microbiome compartments for further characterization, including shotgun metagenomics and single-cell genomics.

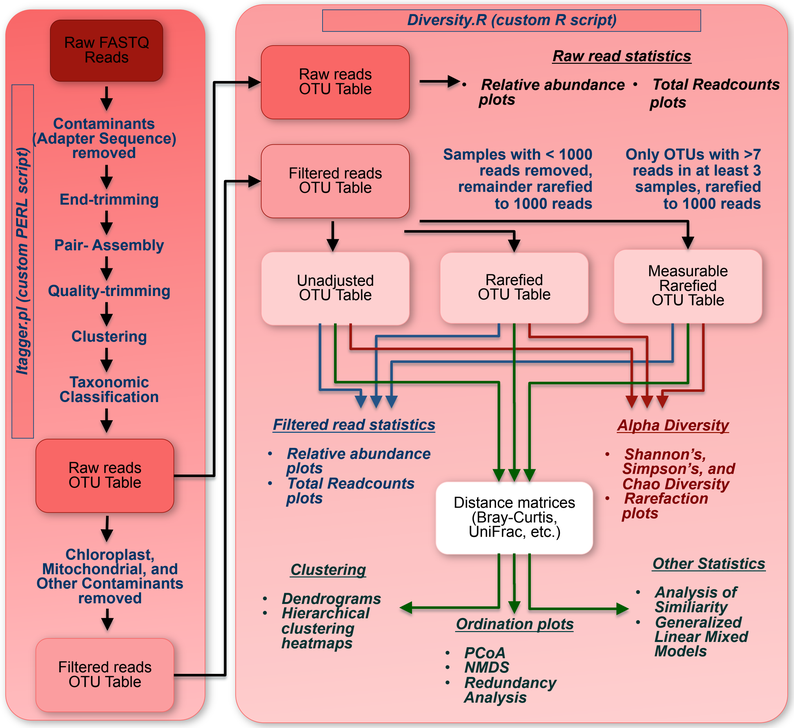

Figure 4. Flow chart for the pipeline of analysis tools used in processing the 16S iTag amplicon data. Initial processing of the MiSeq generated FASTQ reads is handled by a custom PERL script (itagger.pl) and subsequent statistical analyses are performed in the R platform.

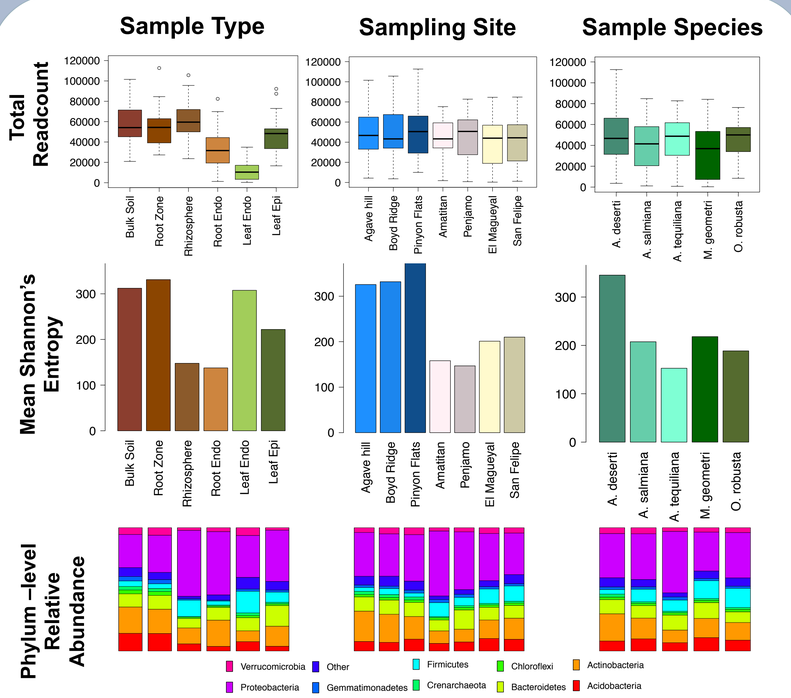

Figure 5. Alpha and beta diversity plots by Sample type, Sampling Site, and Species. Each row of panels (from top to bottom) represent filtered readcounts, Shannon Diversity, and Phylum-level relative abundance.

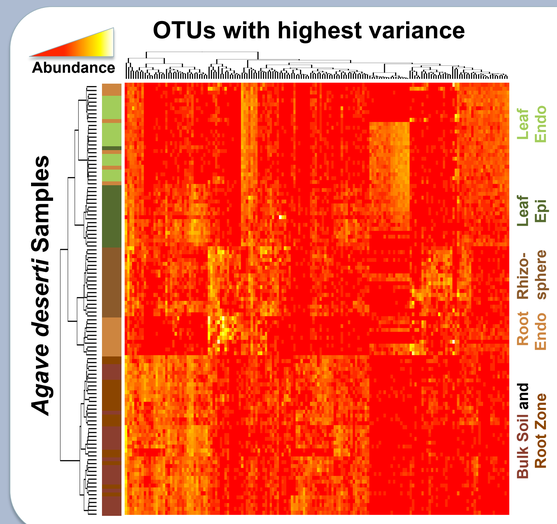

Figure 6. Heatmap of log-abundance of readcounts in all *Agave deserti* samples for OTUs with the top two percent of variance across samples. Samples are ordered by Ward clustering of the Bray Curtis distances, and false colored at left by sample type. Indicator species analysis will be used to determine OTUs correlating with each compartment.

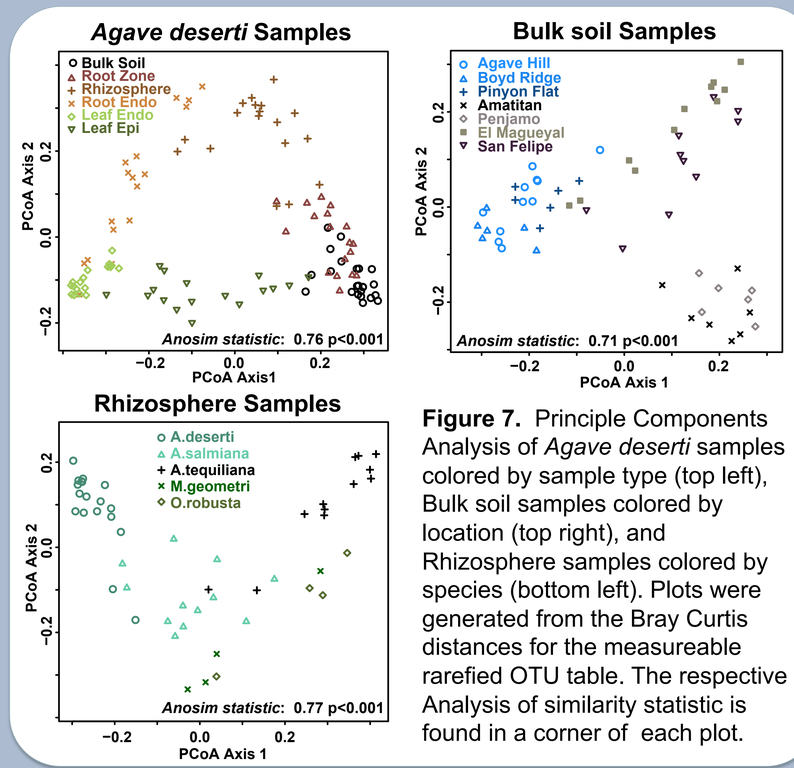

Figure 7. Principle Components Analysis of *Agave deserti* samples colored by sample type (top left), Bulk soil samples colored by location (top right), and Rhizosphere samples colored by species (bottom left). Plots were generated from the Bray Curtis distances for the measureable rarefied OTU table. The respective Analysis of similarity statistic is found in a corner of each plot.

Conclusion: We are investigating and comparing the microbial communities of native and cultivated Agave species in California and Mexico under a panel of different environmental conditions. Our project will expand our understanding of microbial diversity in desert soils, catalog and characterize the microbial factors that contribute to *Agave*'s successful adaptation to the extreme environments of its endemic range. Ultimately, we aim to enable microbiome manipulation aimed at improving the suitability of *Agave* for use in the rapidly growing biofuels industry.

References:

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