Analysis of an Abundant Bacterial Genus in a Leaf Litter Community

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Project Goals: With increasing availability of cultured and uncultured (metagenome-reconstructed and single-cell) genomes, it is now feasible to study the ecology and genetic diversity of specific microbial populations in complex communities. Previously, using 16S rRNA sequencing, we identified *Curtobacterium* (phylum: Actinobacteria, family: Microbacteriaceae) to be the most abundant taxon (~18% relative abundance) in a leaf litter grassland community in Southern California (Matulich *et al.* 2015). Here we analyze shotgun metagenomic libraries (Berlemont *et al.* 2014) at this site and ask: 1) How the abundance of *Curtobacterium*, and more specifically clades within *Curtobacterium*, responded over a two-year period (2010-2012) and across three treatments: drought, increased nitrogen, and control. 2) What is the genetic variation within populations of *Curtobacterium* over time?

We found that traditional characterization of microbial abundance in metagenomic libraries (i.e. MG-RAST) lack the genomic representation in their database to accurately quantify relevant taxa of interest, particularly within soil communities. To address these concerns, we isolated and sequenced 14 *Curtobacterium* genomes (Chase *et al.* 2016) and constructed a robust reference database and phylogenetic analyses into the Microbacteriaceae family. To quantify microbial abundance, we established an approach to extract and analyze single-copy marker genes within the metagenomic data. Normalizing the metagenomic data using 30 phylogenetically conserved marker genes, we estimated that the relative abundance of all Microbacteriaceae ranges from 22.76% to 29.12% across the samples. By placing short metagenomic reads onto this family reference tree, we showed significant strain-specific responses within clades of *Curtobacterium* over time. To validate these results, we then recruited and mapped reads to 4 genomes representing multiple clades. The mapping corroborated the abundance of specific clades within *Curtobacterium*, suggesting clade-specific responses to changing environmental conditions. Mapping of the reads further revealed genomic variation across clades, specifically with regards to the presence and/or absence of particular glycoside hydrolase genes. Together these results show that targeted analysis at fine taxonomic resolution can reveal population-specific characterizations that would otherwise be masked by broad taxonomic designations.

References

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