Title: Comparative Analyses of the Genomes and Secretomes of Ascomycota Fungi Reveal Diverse Functions in Plant Biomass Decomposition.

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http://www.lanl.gov/org/padste/adcles/bioscience/bioenergy-biome-sciences/soil-carbon.php
http://www.lanl.gov/org/padste/adcles/bioscience/bioenergy-biome-sciences/environmental-microbiology.php

Project Goals: The LANL Genomic Science SFA is focused on microbial communities in surface soil horizons and their functional processes that influence soil carbon storage and release. The SFA examines soil carbon cycling under conditions of environmental change to understand the metabolic and ecological roles of fungi and bacteria in surface soils in two important temperate ecosystems – forests and arid grass/shrub lands. In both biomes fungal and bacterial biomass is concentrated in shallow surface soil strata where C and N cycling are major processes. Advancing fundamental knowledge of soil communities within the context of altered environmental regimens will improve our ability to predict and possibly manage ecosystem contributions to global climate. This involves discovery of fundamental principles at different scales that influence the organization, interactions, and response of soil communities.

In arid grasslands and shrublands, the dominant fungi in surface soils are members of the Ascomycota phylum. Their functions in arid soils, where organic matter, nutrients and water are very low or only periodically available, are unknown. Potential roles include seasonal plant biomass decomposition, direct interactions with plants as endophytes or pathogens that induce selective disassembly of plant tissues, or as integral members of cyanobacteria-dominated biological soil crusts. We isolated and taxonomically typed several thousand fungal isolates from an arid grassland. The genomes of five Ascomycota genera that were abundant in multiple microhabitats were sequenced. Their secreted proteomes (secretomes) when grown on different carbon sources (chitin, native bunchgrass or pine wood) were determined in replicated cultures, through a collaborative project with the Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory. Comparisons of the genomes and secretomes of these five fungi have revealed similarities and differences in their secretomes, also with respect to particular carbon growth substrates, that will advance our fundamental knowledge of the roles that soil fungi play in microbial communities.

Ascomycota genomes were assembled using Velvet version 1.2.10 [1], and gene prediction was accomplished using Augustus version 3.0.3 [2]. Protein coding sequences were functionally annotated by BLASTP [3] against the nr database. For each gene, function was automatically assigned based on the top hit using an in-house script. Protein spectral counts (representing protein abundances) were obtained and mapped to predicted proteins in each fungal genome by the EMSL proteomics resource. Spectral count data were averaged across the technical replicates for each fungus and each treatment; the means, standard deviations, standard errors were calculated. Differences in protein expression were assessed by fold change analysis. For each

fungus grown on each carbon substrate, the fold change of the protein counts for each condition was calculated compared to each other condition and pairwise p-values were calculated. The volcano plots below in Figure 1 show some of the results.

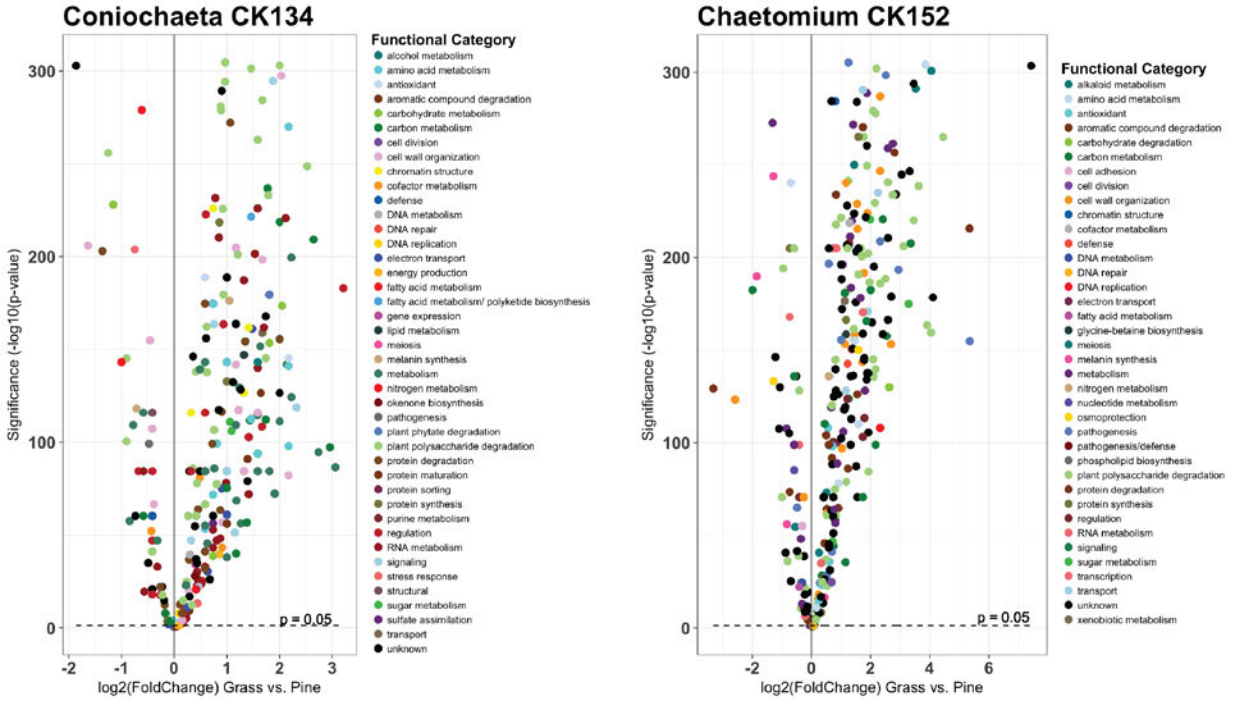

Figure 1. Volcano plots showing results of the Fold Change analysis of protein spectral counts for two of the fungal species sequenced and analyzed for this study: Coniochaeta CK134 and Chaetomium CK152. The plots emphasize the magnitude of differences in protein expression for each fungus grown on grass as the main carbon source compared to pine. The values on the x-axis represent the $\log_2$(FoldChange) and on the y-axis the Significance values represent $-\log_{10}$(p-value). These conversions give the data a nice spread for visualization. The dots represent actual proteins in each genome, colored by the Functional Categories in the legend. NOTE: Functional Categories related to plant biomass decomposition are colored in shades of green. The dots to the left of the 0 line represent decreased expression, while dots to the right of the 0 line represent increased expression, when the fungus was grown on grass compared to pine.

Each of the five fungi secreted a characteristic set of enzymes when grown on individual carbon substrates. While homologs of some secreted proteins were identified in more than one fungal genome, the overall secretome of each fungus showed differences. Results of this study will increase our understanding of the contributions of fungal carbon metabolism to arid land microbiomes and ecosystems, and will contribute to the identification of fundamental principles at different scales that influence the organization, interactions, and response of soil communities to variations in their environment.

References

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