Title: Interspecies Interactions During Chitin Decomposition in a Naturally Enriched Model Soil Microbial Consortium.

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Project Goals: PNNL's Phenotypic Response of Soil Microbiomes SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture. We perform multi-scale examinations of molecular and ecological interactions occurring within and between members of microbial consortia during organic carbon decomposition, using chitin as a model compound. Integrated experiments are designed to confront spatial challenges and inter-kingdom interactions among bacteria, fungi viruses and plants that regulate community functions. These data are used to parametrize individual- and population-based models for predicting interspecies and inter-kingdom interactions. Predictions are tested in lab and field experiments to reveal individual and community microbial phenotypes. Data is captured and shared through an optimized data management pipeline. Knowledge gained will provide fundamental understanding of how soil microbes interact to decompose organic carbon and enable prediction of how biochemical reaction networks shift in response to changing moisture regimes.

Chitin is one of the most abundant carbon and nitrogen sources available in nature. However, its complex chemistry means that complete breakdown of this polymer into constituent n-acetyl-glucosamine (NAG) monomers or other compounds is energetically expensive. As a result, interspecies interactions are central to both the complete breakdown of chitin and dispersion of the carbon and nitrogen within this molecule throughout the soil microbiome. While these interactions are likely critical to organic carbon cycling in soil the molecular mechanisms are not well understood. A better understanding of the role of soil microorganisms in nutrient breakdown is critical if we wish to harness the microbiome to promote plant and ecological health and to gain a better understand of large-scale carbon and nitrogen cycling. Here, we focused on specific species and metabolic interactions that lead to the breakdown of chitin. To study these interactions we developed a naturally evolved simplified model soil consortium (MSC-1) containing ~20 species that were enriched under high chitin conditions (1). Network analysis of this community indicated the potential for several interspecies interactions. Here, we explored specific chitin degrading phenotypes by isolating several constituent strains from MSC-1 and carrying out genomic and phenotypic analyses to decipher species interactions during growth on chitin.

We isolated several axenic strains from MSC-1 and sequenced their genomes to enable us to predict the metabolic capacity of each isolate member. Eight of the isolates were chosen to represent the diversity of the original MSC-1 community and designated as Model Soil Consortium 2 (**MSC-2**). MSC-2 contains *Streptomyces venezuelae*, *Neorhizobium galegaae*, *Dyadobacter fermentans*, *Sphingopyxis fribergensis*, *Ensifer adhaerens*, *Variovorax paradoxus*, a *Sinorhizobium* species. and a *Rhodococcus* species. This collection of species represents six different families, five different orders and three different phyla. Highly abundant and central members of our original MSC-1 consortium, including *Streptomyces venezuelae*, *Ensifer adhearens* and *Rhodococcus* sp., are included in MSC-2. Subsequent genomic analyses of members

of MSC-2 demonstrated that all MSC-2 strains contained a subset of chitinase genes. However, the genetic potential for complete chitin degradation varied between strains. In addition, a subset of strains contained metabolite transporters for chitin breakdown products, including NAG. These observations point to the potential for metabolic complementarity regarding chitin breakdown by MSC-2.

We tested the ability of each MSC-2 strain to grow using chitin as the sole source of carbon. Each strain was grown axenically in M9 media supplemented with ammonium and chitin. Under these conditions we found that only *S. venezuelae, N. galegaae* and *S. fribergensis* were unable to grow on chitin. While the remaining six strains did show an increase in biomass under chitin, the kinetics of each strain's growth were very different. We next examined co-culturing pairs of MSC-2 species to determine if there was greater growth (as measured by optical density) when species were cultivated together vs. axenically. We found that when *S. venezuelae* (a non-grower on chitin) was cultured with *V. paradoxus* (which can grow on chitin) the O.D. of the two-member community was higher than either species grown together. In addition, when two species, both found to be unable to grow on chitin (*S. venezuelae* and *N. galegaae*), were cultured together growth was observed in the co-culture. These observations speak to metabolic interdependencies between species of MSC-2 during chitin degradation. These interdependencies were further explored through fluorescently tagging two of the strains with GFP variants: the *Rhodococcus* and *Sinorhizobium* sps. Fluorescently tagged *Rhodococcus* sp. and *Sinorhizobium* sp. were co-cultured with chitin then treated with NAG-ELF, a chemical tool designed to label functionally active chitinase. Hydrolyzed NAG-ELF was found to be nearly exclusively co-localized with *Rhodococcus* sp. cells, indicating that the *Rhodococcus* sp. chitinases are cell associated and that the *Rhodococcus* sp. is supplying both members with chitin degradation products.

The knowledge gained about interspecies interactions during chitin decomposition that we are obtaining in our model consortia are relative to understanding details of SOM degradation in natural soil ecosystems because chitin is an abundant source of both carbon and nitrogen in soil. It is important to note that all strains of MSC-2 were derived from our naturally evolved MSC-1 so the interactions we observe here likely reflect a subset of those occurring in soil. Our results indicate that even in a community where chitin degradation potential is ubiquitous only a subset of species actually carry out chitin degradation. To provide more mechanistic understanding of these results, we initiated species-resolved community metabolic networks using the KBase pipeline that were based on individual genome-scale metabolic models for the nine MSC-2 isolates. These models revealed that the predicted growth patterns were largely consistent with experimental observations. Our future research is focused on refining details of metabolic and signaling interactions during chitin decomposition, and the role of changes in soil moisture on those interactions.

References:

1. **McClure R, Naylor D, Farris Y, Davison M, Fansler SJ, Hofmockel KS, Jansson JK.** 2020. Development and Analysis of a Stable, Reduced Complexity Model Soil Microbiome. Front Microbiol **11:**1987.

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