Title: Spatiotemporal Mapping of Perturbations in a Naturally Evolved Fungal Garden Microbial Consortium

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Project Goals: This early career research project is dedicated to achieving transformative molecular-level insights into microbial lignocellulose deconstruction through the comprehensive and informative view of underlying biological pathways provided by the integration of spatiotemporal multi-omic measurements (i.e., proteomics, metabolomics, and lipidomics). A focus of this project is to uncover the mechanisms that drive cooperative fungal-bacterial interactions that result in the degradation of lignocellulosic plant material in the leafcutter ant fungal garden ecosystem. This approach will provide the knowledge needed for a predictive systems-level understanding of the fungal-bacterial metabolic and signaling interactions that occur during cellulose deconstruction in an efficient, natural ecosystem.

Abstract Text: Predicting the impact of perturbation on microbial communities relies on a mechanistic understanding of how the community members interact with each other and with the environment. Biological samples, however, are often complex and heterogeneous; thus, it is challenging to detect the variation in community composition and activities across spatial and temporal space. Here, we used a naturally evolved leafcutter ant fungal garden consortium carrying out lignocellulose degradation [1] that was gradually infected by a pathogenic fungus, *Escovopsis*, as a dynamic system to study. Across the vertical gradient of infection, we applied multi-omics (metaproteomics, lipidomics, metabolomics) with an integration of microscale imaging to capture and map shifts in microbial community members and detected activities.

We first curated a reference database from 50 million proteins of known members in the consortium, which were grouped into >24 million clusters based on sequence similarity, to annotate the high-resolution tandem mass spectrometry (MS) spectra with stringent matching criteria. These measurements leveraged a Thermo Fisher Orbitrap Eclipse Tribrid mass spectrometer equipped with a front-end High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) interface to enhance peptide selectivity and sensitivity. A total of 40,119 clusters were detected in the metaproteome data, which unveiled a complex community with relatively high representations of fungal, bacterial, and plant proteins. This was followed by archaeal, and insect proteins. The fungal cultivar, *Leucoagaricus*, has naturally co-evolved with the garden consortium to play a dominant role in breaking down lignocellulose. As the pathogenic fungus, *Escovopsis*, proliferated, the normalized spectral counts of *Leucoagaricus* proteins decreased. Additionally, that of bacterial members were generally increased, suggesting an active community response and potential interkingdom interactions under the impact of fungal infection. Among the most dominant protein clusters, a cluster of *Escovopsis*-specific fatty acid synthase was detected and increased with the infection. A principal component analysis of the

global lipidomic data further indicated that an *Escovopsis* infection could shape the lipid profile of the consortium. Across lipid subclasses, including phosphatic acids (PA), diacylglycerophosphocholines (PC), diacylglycerophosphoethanolamines (PE), and diacylglycerophosphoglycerol (PG), significant trends (adjusted p-value < 0.05) were observed due to infection based on individual fatty acid composition. Plant alpha-linolenic acid (18:3) containing lipids, which we previously found to be molecular indicators of lignocellulose degradation [1], were associated with healthy fungal garden consortium. Conversely, oleic acid (18:1) containing lipids (i.e., PA(18:1/18:1), PC(18:1/18:1), PE(18:1/18:1)) and bacterial odd-chain fatty acid containing lipids (i.e., PC(19:1/19:1), PG(19:1/19:1)) increased with infection. Our metabolomic data captured the depletion of myo-inositol, an essential nutrient for fungi, in regions where *Escovopsis* was highly proliferative [2]. Phosphoric acid with antimicrobial activity against bacteria and fungi was one of the most abundant metabolites detected and gradually increased with the fungal infection. This indicated potential interactions among bacteria and healthy and pathogenic fungi. Leveraging our capabilities in microscale imaging and spatial multi-omics (metabolome and metaproteome), we delineated the spatial distribution of the community members and further mapped the detected activities to those that were co-localized within micron-scale regions.

These spatial temporal multi-omics measurements provided an integrated road map to efficiently harness microbiome data for a better understanding of microbial interactions and community response to a perturbation. In addition, it provided a predictive systems-level understanding of how symbiotic fungal-bacterial metabolic and signaling interactions enable the leafcutter ant fungal garden ecosystem to thrive and degrade lignocellulose in dynamic environments.

References/Publications

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