Development of untargeted metabolomics approaches to study bacterial-fungal co-cultures

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Project Goals: The goal of this project is to understand the complex metabolic interactions between bacterial and fungal partners in mixed culture environments that mimic natural soil microbiomes. Our team is developing a metabolomics pipeline to understand these species-dependent interactions. In the future, we plan to compare metabolism of multiple different co-culture pairs and identify the impact of growth conditions on their interactions using both Gas Chromatography-Mass Spectroscopy (GC-MS) and high-resolution Liquid Chromatography-Mass Spectroscopy (LC-MS) or Desorption Electrospray Ionization-Imaging Mass Spectrometry (DESI-IMS) untargeted metabolomics platforms.

We are developing a data analysis workflow for annotating biologically relevant compounds detected in our samples. We aim to solve two major problems. First, this workflow will be able to process and quantify mass spectra detected using multiple platforms (DESI-IMS, GC-MS, and LC-MS). Second, it will identify conserved metabolites by simultaneously cross-referencing putative targets against multiple spectral libraries, metabolic pathway databases and organismal metabolomes. To the best of our knowledge, no other software possesses these functionalities. This approach will streamline our workflow by eliminating biologically irrelevant targets and aid data interpretation by identifying pathways in our organisms of interest impacted by co-culture conditions. Additionally, the unique ability to simultaneously search multiple related metabolomes is ideal for identifying biologically relevant metabolites in experiments like ours that utilize environmentally derived organisms that may have uncertain phylogeny.

To analyze spatial profiling of extracellular metabolites, we are developing a metabolomic analysis pipeline using DESI-IMS, which uses an electrospray mechanism to ionize metabolites from surfaces under ambient conditions. These methods are being applied to study the interactions of the oxalogenic fungus *Aspergilllus niger* (*A. niger*) with the bacteria *Pseudomonas putida* (non-oxalotrophic*)* and *Cupriavidus oxalaticus* (*oxalotrophic*). Our hypothesis is that the interactions between this fungal and these bacterial strains are dependent

on the spatial distribution of oxalic acid. produced by the fungus and consumed only by the oxalotrophic bacterium. This interaction depends primary on the effect of this low molecular organic acid on the pH of the growth medium. *A. niger* produces oxalic acid, which lowers the pH of the medium. *C. oxalaticus* metabolizes oxalic acid and thereby antagonizes *A. niger* growth by raising the local pH in the vicinity of the bacterial colonies. In contrast, *P. putida* coexists with *A. niger* without altering the pH of the medium. We have developed and are currently testing a DESI-IMS strategy to spatially profile the concentration of oxalic acid and other metabolites by growing 2D co-cultures on semi-permeable membranes placed atop agar plates. Using this approach, we expect to investigate the nature of the metabolic interactions between these species.

By developing new software tools and experimental pipelines, we expect to better understand the microbial metabolic processes that occur at the interface of bacterial-fungal co-cultures.

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