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

Authors: Baltazar Zuniga[1], Amy Zheng[2], Fabio Palmieri[3], Simone Lupini[4], Berkley Ellis[5], Aaron Robinson[6], Julia Kelliher[6], Demosthenes Morales[6], Debora F. Rodrigues[4], Pilar Junier[3], Jamey Young[1,2], Patrick Chain[6]

[1]Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA, [2]Department of Chemical and Biomolecular Engineering,Vanderbilt University, Nashville, TN, USA , [3]Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Emile-Argand 11, 2000 Neuchâtel, Switzerland; [4]Civil and Environmental Engineering, University of Houston, Houston, TX, USA,[5]Chemistry, Vanderbilt University, Nashville, TN, USA, [6] Biosecurity and Public Health Group, Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM, USA

https://genomicscience.energy.gov/research/sfas/lanlbfi.shtml

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 and methods to identify metabolite targets to understand these interactions. These tools are currently being applied to compare the metabolism of multiple different co-culture pairs and identify the impact of growing conditions on their interactions using both targeted and untargeted GC-MS, LC-MS, and NMR methods.

We aim to solve two significant problems using our metabolomics pipeline. First, our pipeline analyzes metabolites detected using GC-MS and LC-MS to produce high-quality tentative compound identifications. Second, our pipeline helps to generate biological interpretations from the compounds we tentatively identify. Our custom metabolomics data processing software performs relative quantification on these compounds and links the identification output to external databases like KEGG for further analysis. The software highlights conserved metabolites by cross-referencing potential targets against related KEGG metabolomes. This approach streamlines the data analysis workflow by prioritizing biologically relevant identifications and annotating the possible functions of these metabolites in relevant organisms, thus allowing us to visualize trends in pathway activation. By developing new software tools and experimental pipelines, we expect to better understand the microbial metabolic processes in fungal:bacterial co-cultures.

We also aim to identify specific metabolite targets of interest found in co-cultures. One such target is a red pigment produced by certain fungi in our culture collection when grown in co-culture with other fungi, thereby inhibiting their growth. Based on the gene clusters present, we hypothesized that the red pigment was bikaverin. We were able to purify the pigment and analyze its structure with NMR spectroscopy, which confirmed the identity of the pigment as bikaverin. We found that bikaverin is only produced in specific co-cultures, leading us to hypothesize that bikaverin could be produced as a defense mechanism to inhibit the growth of competing fungi.

This work was supported by the U.S. Department of Energy, Office of Science, Biological and Environmental Research Division, under award number LANLF59T.