Chemical and Proteomic Profiling of Organic Compounds for Detecting Algal Interactions with Grazers and Commensalistic Bacteria

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Project Goals: The LLNL Bioenergy SFA seeks to support sustainable and predictable bioenergy crop production through a community systems biology understanding of microbial consortia that are closely associated with bioenergy-relevant crops. We focus on host-microbial interactions in algal ponds and perennial grasses, with the goal of understanding and predicting the system-scale consequences of these interactions for biomass productivity and robustness, the balance of resources, and the functionality of surrounding microbial communities. Our approach integrates 'omics measurements with quantitative isotope tracing, characterization of metabolites and biophysical factors, genome-enabled metabolic modeling, and trait-based representations of complex multi-trophic biological communities, to characterize the microscale impacts of single cells on system scale processes. https://bio-sfa.llnl.gov/

Biotic interactions in algal ponds are both affected by and influence the chemical environment surrounding algal cells. As part of our SFA, we aim to disentangle various components of these chemical signals in order to better understand the mechanisms of how biotic interactions influence algal productivity and physiology. Here, we present two studies focused on different aspects—the first highlights the role of trophic interactions, identifying volatiles produced in algal-rotifer interactions, while the second examines algal-bacterial interactions at the molecular level in a model algal species.

Chemical compounds, in particular those defined as volatile, are released by all organisms, and in addition to being byproducts of metabolism, can play roles as signaling molecules, both within and between species. We are interested in detecting, quantifying, and identifying VOCs capable of differentiating microalgal predators. The presented work uses solid-phase microextraction (SPME)-GC-MS to analyze volatiles emitted before and after predator infection in microalgal strains that are currently important to the biofuels community. *Microchloropsis salina* was grown in six- 15 L cultures of chemically-defined, enriched artificial seawater media with high purity gas. VOC profiles were sampled from all cultures after 24-48 hour propagations using 65 µm polydimethylsiloxane-divinylbenzene SPME fibers with 60 minute exposures, and 70 eV electron ionization on a quadrupole mass analyzer. After 48 hours of growth, the marine rotifer, *Brachionus plicatilis*, was added at high density to two of the cultures. VOC profiles and algal counts were monitored for an additional 2-4 timepoints after

inoculation. Including our controls we had four conditions to assist with identification of a rotifer infection: 1) *M. salina*-only cultures, 2) *B. plicatilis*-infected *M. salina* cultures, 3) enriched seawater controls, and 4) SPME fiber blanks. Volatiles were attributed to each condition if meeting the following conservative requirements: 1) presence in <66% of replicates at a timepoint, 2) greater than 10x abundance of corresponding abundance in media-only control. Subsequent characterization, alignment, and preliminary identifications were made through analysis of deconvoluted experimental spectra with comparisons to the NIST14 spectrum database (Match>70%) and retention index matching.

One main finding was that the addition of *B. plicatilis* to healthy cultures of *M. salina* produced an abundance of tentatively identified carotenoids, such as trans β-ionone and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone, which may indicate algal lysis. Concurrent to VOC analysis, daily measurements of algal density confirmed that rotifer-inoculated cultures displayed decreased live algae counts relative to the uninoculated controls. The peak areas of the carotenoid biomarkers were observed to increase when rotifers had consumed greater amounts of algae. Application of multivariate statistical analyses to these datasets are in-progress. Ongoing experiments include work with the closely-related algal species *Nannochloropsis gaditana* and *Nannochloropsis oceanica*, additional rotifer and chytrid predators, and evaluating biomarker targets in complex, open-environment microalgal systems. Our work aims to increase the breadth and depth of reported algal and rotifer-specific VOCs, providing a tool to better define the chemical environment of microalgal ponds.

In parallel, we are also investigating a model green algae, *Chlamydomonas reinhardtii,* and its metabolic interactions with an actinobacterium, *Arthrobacter* sp. P2b. In order to gain a mechanistic understanding of the physiological effects on algal metabolism caused by chemicals released by heterotrophic bacteria, we are using a simple model system of one bacterial mutualist to ultimately understand the complex chemical and biotic interactions occurring in ponds at the molecular and gene level. We observed that both co-culturing with P2b, and P2b cell-free spent media enhanced algal chlorophyll content, biomass, and cell size, suggesting a beneficial or commensal interaction between the two species. In order to determine putative gene pathways involved in *C. reinhardtii* increased biomass, we compared global protein expression between co-cultures and monocultures of each species, as well as the algal monoculture incubated with the P2b cell-free spent media, at two time points. We find that several factors may be involved in the effect of P2b on *C. reinhardtii* physiology, influencing pathways related to cell cycle regulation.

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