Characterizing algal metabolites and their role in biotic interactions

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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.

It has become increasingly clear that complex metabolic interactions between algae and their associated microbes influence algal physiology and growth. The suite of metabolites produced and exchanged between algae and bacteria are potentially important mediators of these interactions. Through a series of experiments, we set out to profile the intracellular, dissolved, and volatile metabolites produced by a diverse suite of microalgae.

To begin to characterize the diversity of algal metabolite secretion, we used an LCMS metabolomics analysis to compare the extracellular and intracellular metabolite profiles of phylogenetically diverse algal strains: the freshwater algae *Chlamydomonas reinhardtii*, the saltwater algae *Microchloropsis salina*, *Desmodesmus* sp. strain C406, which can grow in either fresh or saltwater, and the diatom *Phaeodactylum tricornutum*. The metabolite profiles of the intracellular and extracellular metabolites were quite distinct, suggesting that the detected exometabolites are unlikely to have originated from lysed cells. Exometabolite profiles also differed significantly between algae, with a few shared metabolites. Interestingly, when *Desmodesmus* was grown in freshwater versus saltwater, the metabolite profiles of both the intracellular and extracellular metabolites changed significantly. This suggests that along with a broad phylogenetic diversity, secreted as well as internal metabolites are modulated in response to external environmental cues and physiological state.

To follow up on the dynamics of exometabolite production, we focused on *P. tricornutum* throughout a growth cycle, and identified metabolites present at various stages of algal growth. The identified metabolites included phytohormones, several B-vitamins, and B-vitamin derivatives. There was a marked progression of metabolite composition and accumulation over time in both the intracellular and extracellular metabolite profiles. Within the extracellular metabolite pool, some metabolites were produced early in growth but did not continue to accumulate in later in growth, while others were at low or undetectable levels in early time points and only accumulated in late log and stationary phase. This suggests that *P. tricornutum* metabolite secretion is both dynamic and regulated to some degree by growth stage. We are currently investigating the potential of these extracellular metabolites from *P. tricornutum* to influence the growth of bacteria from our isolate library.

In parallel, in collaboration with Sandia National Laboratories we are identifying chemical and genetic signatures of microbial communities that are either indicative of pond health or the presence of algal grazers, parasites and pathogens. Towards this end, we have used both solid-phase microextraction (SPME) and thermal desorption (TD) coupled with gas chromatography-mass spectrometry (GC-MS) to survey the production of algal volatile organic compounds (AVOCs) by several strains of microalgae in the presence and absence of algal grazers and other deleterious species and stress conditions. The addition of the grazer, *Brachionus plicatilis,* to healthy cultures of *M. salina* led to emissions of the carotenoid degradation products, trans-β-ionone and β-cyclocitral which increased with rotifer consumption of algae. In addition to carotenoid breakdown products we have identified additional AVOCs that are indicative of grazer activity or cell disruption in algal mass cultures. Our results indicate that specific AVOCs may be early indicators of grazing or other algal disruption providing a useful tool to monitor algal biomass production and prevent pond crashes. Our results also shed light on the complex suite previously uncharacterized volatile organic compounds in produced as a result of trophic interactions in these diverse algal cultures.

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