

## Characterizing Algal Exo-Metabolites and Their Impacts on Algae and Associated 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.**

Complex metabolic interactions between microalgae and their associated microbial communities influence algal physiology and growth, with implications for both bioenergy production and global biogeochemical cycling. The suite of extracellular metabolites produced and exchanged between algae and bacteria are potentially important mediators of these interactions. Here, we present research taking the first steps toward a more global profile of algal exo-metabolites and their role in algal-bacterial interactions. We set out to determine: (a) whether phylogenetically distinct algal taxa had distinct exo-metabolomes, (b) how specific algal exo-metabolites affect algal growth, (c) whether algal exo-metabolomes have predictable dynamics and (d) the effect of specific exo-metabolites on phycosphere-associated bacteria.

We profiled the extracellular and cell associated metabolites of four phylogenetically diverse algal strains using liquid chromatography tandem mass spectrometry (LC-MS/MS). We characterized metabolites from axenic cultures of the freshwater green alga *Chlamydomonas reinhardtii*, *Desmodesmus* sp. strain C406, which can grow in either fresh or salt water, the saltwater algal *Microchloropsis salina*, and the diatom *Phaeodactylum tricornutum*. The metabolite profiles of these algae differed significantly, reflecting their phylogenetic diversity. For each alga, the extracellular and cell associated metabolite profiles were also distinct, indicating that the extracellular metabolites were not primarily the product of cell lysis, but instead were more likely excreted or produced extracellularly. We used the global natural products social networking (GNPS) pipeline to generate putative identifications for some

metabolites. Putatively identified metabolites included known phytohormones and plant growth promoters, as well as signaling molecules. We hypothesized that some of these metabolites might be involved in intra-algal signaling, and thus might affect algal physiology and growth. We investigated the impacts of 10 metabolites enriched in the extracellular pools of one or more algal strains on each of the axenic algal cultures. We demonstrated that some metabolites significantly increased total chlorophyll in some algal cultures, while other metabolites decreased chlorophyll.

We sought to further characterize the temporal dynamics of the exo-metabolome of the model diatom *P. tricornutum* through its growth cycle. Using a targeted LC-MS/MS analysis, we identified 60 metabolites, including phytohormones, several B-vitamins and B-vitamin derivatives, and a suite of aromatic organic acids. We found that different sets of metabolites accumulated in the extracellular metabolite pool during the lag, early log, late log, and stationary growth phases, suggesting that there is growth phase dependent regulation of the exo-metabolome. Based on our analysis, we selected a set of 15 *P. tricornutum* exo-metabolites for further study to better understand how these metabolites may be involved in algal-bacterial interactions. The selected metabolites had different accumulation patterns across the range of *P. tricornutum* growth phases. We investigated the ability of 12 phycosphere-associated bacterial isolates to grow in a defined saltwater medium with each of these metabolites provided individually as the carbon source. We found that several of the metabolites could support growth of some bacterial isolates. The patterns of substrate use differed between bacterial isolates, suggesting that algal exo-metabolites could modulate microbial communities by selectively supporting the growth of different bacterial strains in the surrounding communities. Together, these results reveal the broad diversity of algal exo-metabolites, which are likely to have distinct functions in governing algal microbiomes and algal ecophysiology.

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