

**Title:** Dynamic *Phaeodactylum tricornutum* Exometabolites Shape Surrounding Bacterial Communities

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**Project Goals:** Algal and plant systems have the unrivaled advantage of converting solar energy and CO<sub>2</sub> into useful organic molecules. Their growth and efficiency are largely shaped by the microbial communities in and around them. The  $\mu$ Biospheres SFA seeks to understand phototroph-heterotroph interactions that shape productivity, robustness, the balance of resource fluxes, and the functionality of the surrounding microbiome. We hypothesize that different microbial associates not only have differential effects on host productivity but can change an entire system's resource economy. Our approach encompasses single cell analyses, quantitative isotope tracing of elemental exchanges, 'omics measurements, and multi-scale modeling to characterize microscale impacts on system-scale processes. We aim to uncover cross-cutting principles that regulate these interactions and their resource allocation consequences to develop a general predictive framework for system-level impacts of microbial partnerships.

**Abstract Text:** Algal-bacterial interactions in phycosphere microbial communities have important implications for the stability and productivity of algal biofuel systems. However, the role of exometabolites in mediating these interactions and establishing microbial community structure are not well understood. We set out to characterize exometabolites from the model diatom *Phaeodactylum tricornutum* and investigate the role of those exometabolites in driving the composition of surrounding microbial communities.

First, to characterize the impacts of algal exudates on microbial community composition, we compared the compositions of bacterial communities originating from an algal enrichment (1) grown under three conditions: with alga (*P. tricornutum*) present, with algal exudates (spent medium from axenic *P. tricornutum* growth), and without any algal exudates (alga free controls). Using amplicon sequencing based microbial community analysis, we found that communities grown with algal spent medium were intermediate in composition between those grown with the alga present and algae free controls. Both algal spent medium and algal presence drove shifts in relative abundances compared to alga free controls. Thus, although the communities grown on algal spent medium and with algal were distinct, our results indicate that algal exudates, and the exometabolites present in them, are important for shaping surrounding bacterial communities.

To identify metabolites that could be driving microbial community composition, we used an LC-MS/MS metabolomics analysis to characterize the metabolite composition of the algal spent medium at different growth stages. We identified 58 metabolites produced by *P. tricornutum*

including organic acids, vitamins, amino acids, and nucleotides and derivatives of several of these. Exometabolite production was dynamic, with different metabolites accumulating during different algal growth phases. To investigate the potential role of specific metabolites in shaping microbial communities, we chose 14 identified exometabolites and tested their ability to selectively support the growth of 12 individual bacterial isolates from algal associated enrichment communities (1) and seawater. Of the 14 metabolites tested, three (4-hydroxybenzoic acid, shikimic acid, and hydroxyphenylacetic acid) supported growth of at least one of the bacterial isolates. However, each metabolite supported growth of a subset of the isolates, suggesting that increased levels of these metabolites could selectively increase growth of some bacteria and not others within a community.

To better understand the roles of specific exometabolites in the context of a complex microbial community, we extended the microbial community experiment above to compare community compositions in response to addition of two specific algal exometabolites: one which supported the growth of 4 out of 12 bacterial isolates (4-hydroxybenzoic acid), and another, lumichrome, a vitamin derivative which did not support detectable growth but which has been shown to affect *P. tricornutum* growth (2). Exogenous additions of both metabolites had significant impacts on bacterial community compositions, both with and without *P. tricornutum* present. Addition of 4-hydroxybenzoic acid led to substantial increases in relative abundances of two amplicon sequence variants (ASVs) which were identical to the 16S-V4 sequences of bacterial isolates that grew on 4-hydroxybenzoic acid in the experiment above. The addition of lumichrome also impacted the relative abundances of ASVs, but the responses differed, and were generally lower in magnitude than those to 4-hydroxybenzoic acid. Our work demonstrates the importance of specific algal exometabolites in driving microbial community composition and suggests that algal exometabolites have distinct effects on microbial communities, in part as a function of their bacterial nutritional capability.

### References/Publications

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2. Brisson VB, Mayali X, Bowen B, Golini A, Thelen M, Stuart RK, Northen TR. 2021. Identification of effector metabolites using exometabolite profiling of diverse microalgae. *mSystems* 6, e00835-21.

**Funding Statement:** *This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 and supported by the Genome Sciences Program of the Office of Biological and Environmental Research under the LLNL microBiospheres SFA, FWP SCW1039. LLNL-ABS-830258.*