Impacts of physical proximity and metabolite diffusion on algal-bacteria interactions

Hyungseok Kim[1]* (hskimm@mit.edu), Jeffrey A. Kimbrel,[2] Ty Samo,[2] Jessica Wollard,[2] Christina Ramon,[2] **Rhona Stuart**,[2] Peter K. Weber,[2] Cullen R. Buie,[1] and Xavier Mayali[2]

[1]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA; [2]Physical and Life Science Directorate, Lawrence Livermore National Laboratory, Livermore, CA.

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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.

Metabolic interactions between biofuel-producing microalgae and heterotrophic bacteria (the algal microbiome) dominate the elemental cycling of these highly productive phototrophic ecosystems, where bacteria can incorporate up to half of the photosynthetically fixed carbon. These metabolic interactions occur at the scale of single microbial cells, but most methods to investigate these phenomena are not optimized for measurements at this small scale, making it challenging to capture interactions mediated by diffusion and/or cell attachment. To address this, we have taken two approaches. First, in collaboration with MIT, we have built a co-culture hydrogel microplate for algae and bacteria where the microalgal culture, *Phaeodactylum tricornutum*, can grow to a concentration ~20 times greater than batch conditions, and different bacterial populations can be incubated at different distances from the algal source of organic matter (Fig. 1). Second, using fifteen model bacterial co-cultures growing with *P. tricornutum*, we quantified bacterial impact on algal growth under different nutrient and light conditions, and organic algal-derived C and N incorporation at the single cell level using isotope tracing and NanoSIMS.

Using the co-culture microplate, our experiments quantified how two algal-associated bacteria responded at the population level to the presence of the algae grown at different distances. We also examined the impact of inorganic nutrients and algal organic matter diffusion on bacterial community structure development. These data, combined with a diffusion-based numerical derivation, help us propose a new model of bacterial responses towards algal exudate as a function of algal growth state. Concurrently, using laboratory batch cultures and NanoSIMS, we found surprising variability in the net C and N fluxes between algae and bacteria, which led us to identify a continuum of metabolic interactions from bacterial strains with high levels of C and N exchange through the metabolism of complex organic matter, to strains with low to undetectable exchange. These data challenge the generally accepted view that algal-associated bacteria require algal-derived organic matter for growth, particularly organic nitrogen. We also provide direct evidence of bacterial incorporation of nitrate, suggesting that algal-associated bacteria could switch from commensal or mutualists to competitive under N limitation, and confirming hypotheses generated by our microplate hydrogel experiments.

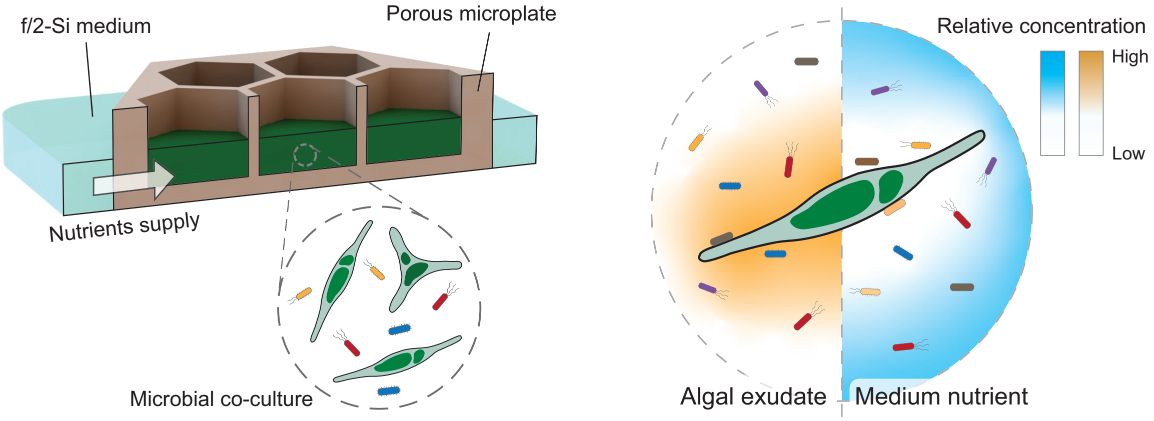

Figure 1. (Left) Schematic of a porous microplate system to co-culture algae and bacteria. (Right) Schematic of a proposed model of nutrient gradients in a phycosphere with bacterial responses.

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