

Categorizing metabolic exchange and signaling reveal distinct mechanisms of mutualistic algal-bacterial 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.

To gain a mechanistic understanding of autotroph-heterotroph interactions in algae-dominated biofuel-producing systems, we are using the saltwater diatom *Phaeodactylum tricornutum* as a model. We have isolated 15 strains of heterotrophic bacteria from outdoor *P. tricornutum* raceway ponds which span the taxonomic diversity of microbial communities associated with saltwater microalgae, numerically dominated by Gammaproteobacteria, Alphaproteobacteria, and Bacteroidetes. We maintain these bacteria as stable co-cultures with *P. tricornutum* in laboratory batch mode without external inputs of organic material, using only light and inorganic nutrients to sustain growth. We are using microscopy, genomics and transcriptomics to investigate what functional genes are required, and in some cases, expressed for bacterial attachment and interaction with *P. tricornutum*. In addition, stable isotope probing combined with imaging via nano secondary ion mass spectrometry (NanoSIMS) enables us to quantify the cell-specific activities of both algae and bacteria when incubated together under different conditions.

Using ¹³C and ¹⁵N labeling of high molecular weight algal excreted organic matter (collected via solid-phase extraction) added back to algal-bacterial co-cultures, we have quantified bacterial incorporation and remineralization of organic C and N back to the algal cells. Surprisingly, we found that incorporation of algal organic matter is not universal among our isolates, suggesting the algal-associated bacteria can be divided into two categories. The first category comprises bacteria with strong metabolic interactions with their algal host, that incorporate algal-organic C and N and generally (but not always) remineralize N (and sometimes C). The second category

comprises bacteria with weak or no metabolic interactions with their algal host, that do not incorporate much algal-excreted organic matter. Some of these bacteria, in fact, are able to incorporate inorganic nitrogen and unknown sources of C. Particularly noteworthy is that bacterium *Marinobacter* strain 3-2, an algal mutualist, generally belongs to the second category, suggesting that mutualistic bacteria are not necessarily taxa with strong metabolic exchange with their algal host. Another bacterium, *Algoriphagus* strain ARW1R1, exhibits strong metabolic exchange with *P. tricornutum* but is only mutualistic under low light and high nutrient conditions, which is partially mediated by the metabolism of the small molecule nicotinamide (vitamin B3). From profiling these different types of bacteria and comparing with other metrics of influence on algal physiology, a framework has emerged for different grouping of bacterial associates of *P. tricornutum*, which has implications for our understanding of niche partitioning and diversity maintenance of algal-associated microbial communities.

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