Characterization of microalgal-bacterial symbioses at the microscale to examine association-dependent remineralization processes at the single-cell level

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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.

As part of our investigations into the effects of algal-associated bacteria on biomass production (Samo et al. 2018), we have continued studying the interactions between the model diatom *Phaeodactylum tricornutum* CCMP2561 and individual bacterial strains isolated from outdoor raceways, including strains that are numerically abundant and can be found as phycosphere-associated. Our overarching goals are to: 1) examine the role of physical interactions (i.e., attachment) in the remineralization of excreted dissolved organic matter as well as 2) leverage and develop a suite of new approaches to quantify and monitor the underpinnings of these associations.

To quantify the remineralization of *P. tricornutum* organic exudates by bacteria and subsequent transfer back to algal cells as inorganic C and N, we employed a high-resolution stable isotope imaging approach (nanoSIP; Pett-Ridge and Weber 2012). In a previous experiment performed with *P. tricornutum* and a *Marinobacter* strain, we found that the bacteria incorporated ^{15}N-labeled exudates and transferred significantly more ^{15}N-labeled compounds to the algal host compared to the algae grown axenically. Here, we collected isotope-labeled exudates released from axenic algae grown in ^{13}C-bicarbonate ($NaH^{13}CO_3$) and ^{15}N-nitrate ($Na^{15}NO_3$) using solid phase extraction, fed these exudates to individual co-cultures of *P. tricornutum* and 16 bacterial genera, and incubated for 24 h. We examine the rate of transfer of both C and N from bacteria to algae, providing relative benefit metrics of each bacterial genus to algal nutrient requirements. Additionally, in collaboration with PNNL, we are beginning to investigate how attachment of one of these bacterial isolates, *Marinobacter* sp. 3-2 (*Marinobacter*), to *P. tricornutum* cells

progresses over time and facilitates the exchange of compounds modulating these symbioses (Fig. 1).

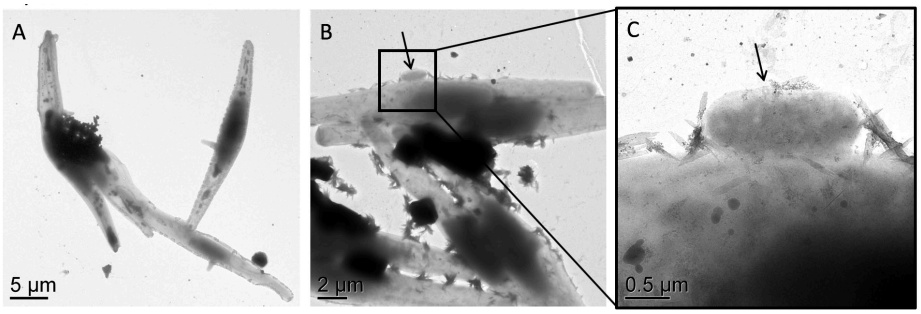

Figure 1. CryoTEM images depicting close associations between P. tricornutum *and* Marinobacter *sp. 3-2. A) View of four individual* P. tricornutum *cells. B) Enhanced view of a single* Marinobacter *cell attached to the surface of* P. tricornutum*, with C) depiciting a highly zoomed acquisition of the bacterium and its orientation on the algal cell.*

In order to better understand *P. tricornutum*-bacterial interactions at the molecular and genetic level, and identify putative genes involved in beneficial interactions, we also conducted global protein expression profiling. We compared *P. tricornutum* axenic cultures with two *P. tricornutum*-bacterial co-cultures, both during mid-exponential growth. The two bacterial strains have distinct interactions with *P. tricornutum*. The first includes the above *Marinobacter* isolate, which is capable of attachment, and enhances growth and single cell carbon fixation. The second, *Rhodobacteriaceae* sp. PT6CLA, does not attach to *P. tricornutum* nor provide any significant growth or carbon fixation enhancement. The two bacterial strains also exhibit distinct growth patterns in co-culture, with *Marinobacter* maintaining low, consistent abundance relative to *P. tricornutum,* and PT6CLA increasing exponentially with *P. tricornutum* growth. Proteomic profiling comparing these co-cultures with each other and the axenic culture revealed a number of putative pathways involved in algal-bacterial interactions, highlighting the multifaceted nature of the interaction.

Our findings have implications for how we understand nutrient cycling at the microscale and highlight the influence of pairwise interactions between heterotrophic bacterial symbionts and their photosynthetic algal hosts on bulk biogeochemical cycling. By combining multiple methods, we find that molecular, biogeochemical, and physiospatial factors are all critical to our understanding of algal-bacterial interactions at the microscale and inform efforts to extrapolate these observations to the community scale using numerical and conceptual models.

References

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