Nanoscale Stable Isotope Tracing and Compound-Specific Radioisotope Analysis to Investigate Metabolic Interactions between Bacteria and Biofuel-producing Algae

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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 investigate the influence of algal-associated bacteria on photosynthetic biomass production, we are examining interactions between the model diatom *Phaeodactylum tricornutum* CCMP2561 and its associated microbiome. Using an enrichment process on outdoor pond samples of *Phaeodactylum* sp., we obtained simplified bacterial communities as well as single bacterial isolates that influence the growth of *P. tricornutum* in the laboratory under different temperature, light, and nutrient concentrations. We aim to better understand 1) the role of physical interactions (i.e., attachment) in the remineralization of excreted dissolved organic matter for improved algal productivity as well as 2) develop new approaches to measure the production and fate of metabolites that influence algal productivity (e.g. plant growth hormones and vitamins).

To address the first goal, we are using the Nano-SIP approach (Pett-Ridge and Weber, 2012) to examine cell-specific carbon fixation and carbon remineralization. This involves adding stable isotope labeled substrates followed by NanoSIMS quantification of isotope incorporation at the single cell level (Figure 1). Using ^{13}C labeled bicarbonate addition experiments, we quantified the carbon fixation activities of the algal cells when incubated on their own and when co-incubated with different bacterial strains. In the co-cultures, the high spatial resolution of the NanoSIMS enabled us to identify algal cells with and without attached bacteria, as well as quantify the amount of fixed carbon incorporated by both attached and non-attached bacteria. Our data show that a subset of the bacterial isolates can increase the carbon fixation activities of *P. tricornutum* cells, and that bacterial attachment plays a critical role in this process, likely

through remineralization of excreted fixed C back into CO_2. We further quantified the remineralized C and N incorporated by the algal cells through similar NanoSIMS analyses of one bacterial-algal co-culture (strain Pt3-2) incubated with ^{15}N and ^{13}C labeled algal exudate.

To address the second goal of metabolite tracing, we are using Parallel Accelerator Mass Spectrometry- Molecular Mass Spectrometry (PAMMS), which involves incubation of cells with ^{14}C-labeled precursors and parallel analysis by liquid chromatography time of flight mass spectrometry (LC-TOFMS) and accelerator mass spectrometry (AMS). This allows the simultaneous interrogation of metabolites, providing identification and quantitation of ^{14}C signal. One well characterized mechanism of algal growth promotion by bacteria centers on the compound indole-3-acetic acid (IAA). Using algal-produced tryptophan, bacteria produce and release this metabolite, which is incorporated by algae and stimulates growth by upshifting cell cycle progression (Amin et al., 2015). To examine this pathway, we carried out separate incubations with ^{14}C-tryptophan in two bacterial strains isolated from *Phaeodactylum* ponds to trace both the intracellular and excreted fate of this precursor molecule in the bacterial metabolomes. There was no evidence for excretion of tryptophan-derived metabolites by these two bacterial cultures. In one of the strains (*Marinobacter* sp. 19DW), the radiolabel was not traced into any metabolites, showing that it remained as tryptophan or was directly incorporated into protein. In *Algoriphagus* sp. ARW1R1, however, the radiolabel was traced into several unidentified metabolites as well as into 2-amino-3-carboxymuconate semialdehyde, an intermediate of the niacin vitamin biosynthetic pathway. These results show that *Algoriphagus* metabolizes tryptophan into compounds potentially influencing algal health and productivity.

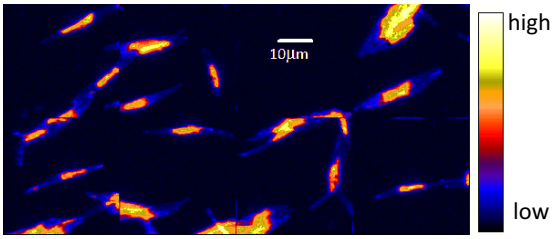

FIGURE 1: NanoSIMS $^{13}C/^{12}C$ isotope map of *Phaeodactylum* cells incubated with bacterial isolate *Muricauda* ARW7G5W

Our data suggest that bacterial taxonomic identity, including the presence of specific biosynthetic pathways, as well as physical proximity to the algal cells play critical roles in mediating the growth of algae. Future plans aim to incorporate such data into metabolic and trait-based modeling efforts to ultimately predict the impact of bacterial metabolism on biofuel production.

References

Amin, S., Hmelo, L., Van Tol, H., Durham, B., Carlson, L., Heal, K., Morales, R., Berthiaume, C., Parker, M., and Djunaedi, B. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522**,** 98-101.

Pett-Ridge, J., and Weber, P.K. (2012). "NanoSIP: NanoSIMS applications for microbial biology," in *Microbial Systems Biology: Methods and Protocols,* ed. A. Navid. Humana Press.

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