NanoSIMS Isotope Imaging and Genome-enabled Metabolic Modeling to Investigate Algal-Bacterial Interactions in Biofuel-Producing Communities

Xavier Mayali[1] (mayali1@llnl.gov), Ty Samo[1], Daniel Nilson[1], Anthony Siccardi[2], Todd Lane[3], Jeffrey Kimbrel[1], Patrik D'haeseleer[1], Marc Griesemer[1], **Ali Navid**[1], Carol Zhou[1], **Peter K. Weber**[1], **Jennifer Pett-Ridge**[1]

[1]Lawrence Livermore National Laboratory, Livermore CA; [2]Texas A&M Corpus Christi, [3]Sandia National Laboratory, Livemore CA

Project Goals: The LLNL Biofuels SFA seeks to support robust and sustainable microalgae fuel production through a systems biology understanding of algal-bacterial interactions. We hypothesize that by understanding the factors that control cellular physiology and biogeochemical fluxes in and out of algal cells, particularly through the phycosphere, we can advance the efficiency and reliability of algal biofuel production. Our research includes studies of beneficial traits of phycosphere-associated bacteria, systems biology studies of model algae, and genome-enabled metabolic modeling to predict the interspecies exchanges that promote algal growth, lipid production and healthy co-cultures. Our overall goal is to develop a comprehensive understanding of complex microbial communities needed to advance the use of biological properties for practical energy production.

To better understand how bacteria promote the growth of microalgae, we are investigating algal-bacterial interactions in batch laboratory cultures of two algal species (*Nannochloropsis salina* CCMP 1776 and *Phaeodactylum tricornutum* CCMP 2561). Outdoor algal pond samples from Corpus Christi, Texas, were obtained and the free-living bacterial fraction (< 1 micron) was added to previously bacteria-free (axenic) cultures to create primary enrichments. These samples were further enriched for algal cell surface (phycosphere) associated bacteria by collecting the algal cells, washing and creating secondary enrichments via dilution cultures. Multiple enrichments were investigated for the ability of attached bacteria to influence the cell-specific carbon fixation rates of the microalgal cells using NanoSIMS and isotope probing (NanoSIP[1]). Cultures were incubated with ^{13}C bicarbonate to track inorganic C fixation and ^{15}N-leucine to track bacterial production and then analyzed with LLNL's NanoSIMS 50 to quantify individual cell's isotope incorporation. Most primary bacterial enrichments did not have significant effects on microalgal cell specific C fixation rates, and attached bacteria generally exhibited faster growth than free-living bacteria. However, the majority of secondary enrichments exhibited the opposite pattern: algal cells with attached bacteria had increased cell specific C fixation rates, and free-living bacteria had increased growth rates compared to phycosphere-attached bacteria. Current and future efforts will include the isolation of single phycosphere-associated bacterial species with probiotic effects, and whole genome sequencing to enable metabolic modeling and an understanding of the genetic basis for observed probiotic effects.

Algal-bacterial mutualism involves the secretion and exchange of nutrients and vitamins, as well as signaling molecules, into the phycosphere. The ability of an individual microalgal cell or bacterium to utilize these extracellular chemicals can be computationally modeled to predict optimal conditions for incorporation of these

compounds into increased cellular biomass or bioproducts. High-quality modeling, however, requires comprehensive and accurate genome annotation, which depends on high-quality sequencing and genome assemblies, in addition to highly curated and validated functional annotation databases. Our analysis pipeline combines high-quality draft genomes from JGI, with our in house functional annotation pipeline to map enzymes to metabolic pathways. The functional annotation includes JGI IMG and RAST annotations supplemented with the LLNL PSAT tools[2]. In our current work, we are comparing and integrating multiple functional annotation and metabolic reconstruction tools to increase the number of reactions and metabolites predicted from a genome. In future work, curated models will be used to validate isotope exchange determined by NanoSIMS, as well as to rapidly identify potential new routes of nutrient exchanges for algal-bacterial interactions to be experimentally tested.

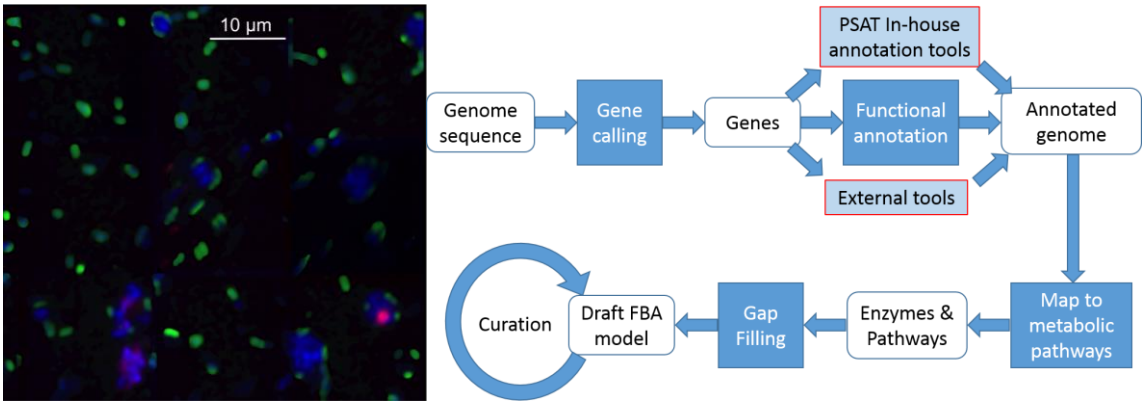

Figure 1. Left: False color stitched NanoSIMS images of *N. salina* secondary enrichment culture: green = heterotrophic ^{15}N leucine incorporation, red = autotrophic ^{13}C carbon fixation, blue = biomass; Right: current pipeline for functional annotation to obtain flux balance analysis models from draft genomes.

References

1 Pett-Ridge, J. & Weber, P. K. in *Microbial Systems Biology: Methods and Protocols* (ed Ali Navid) 375-408 (Humana Press, 2012).

2 Leung E, Huang A, Cadag E, Montana A, Soliman JL, Zhou CLE (2016) Protein Sequence Annotation Tool (PSAT): a centralized web-based meta-server for high-throughput sequence annotations. BMC Bioinformatics. **DOI:** 10.1186/s12859-016-0887-y

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