Isotope tracing and phylogenetic composition of simplified bacterial communities conferring growth and biomass enhancements to biofuel-producing microalgae

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Project Goals: The LLNL Biofuels SFA seeks to support robust and sustainable microalgae fuel production through a systems biology understanding of algal-bacterial interactions. 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 the microbial and metabolic factors that control cellular physiology and biogeochemical fluxes in and out of algal cells, particularly through the phycosphere.

URL: http://bio-sfa.llnl.gov/

Mutualistic algal-bacterial interactions can arise when bacteria provide metabolically beneficial substances in exchange for energy-dense algal compounds. We have studied these positive exchanges in closely interacting algal-bacterial cultures, and simplified algal-attached bacterial communities developed via culture enrichments. We monitored the algae for elevated growth and biomass characteristics and quantified C and N exchanges with both bulk and single-cell approaches (using nanoscale secondary ion mass spectrometry (NanoSIMS)). Through successive rounds of culturing, we have established over 100 stable algal-bacterial co-cultures that exhibit increased algal productivity (as determined by chlorophyll fluorescence), and we have isolated several dozen phycosphere-attached bacteria.

We used two model biofuel-producing microalgal strains, *Phaeodactylum tricornutum* (*Pt*) and *Nannochloropsis salina* (*Ns*), to enrich for growth-promoting bacteria acquired either from the coastal Pacific Ocean or established algal raceway ponds in Texas. After enriching for phycosphere-attached bacteria, microbial communities (characterized by 16S rDNA gene sequencing) exhibited a higher abundance of some bacteria that were rare in the source communities, and lower abundance of bacteria with an exclusively "free-living" lifestyle (those abundant in the culture supernatant but below the limit of detection in the washed algal filtrate). The mechanisms leading to these community composition changes vary, and appear to depend on the source inoculum. Replicates from some sources had nearly identical emergent communities due to a consistent increase in bacteria that were rare in the source communities. However, in emergent communities from other sources, stochastic losses result in increased heterogeneity of community composition among the replicates. Inoculating a source community with either *Pt* or *Ns* had similar results, in both cases the host exerted strong selection to shape the microbial community. Compared to the original source communities, enrichments led to significant increases in the *Rhodobacterales*, most notably *Loktanella, Ruegeria* and *Labrenzia* genera. Additionally, *Rhodobacterales, Sphingobacterales* and *Alteromonadales* genera were

found to be significantly enriched in the phycosphere-attached over the free-living fractions.

In the next phase of this project, we established twelve co-cultures of *Pt* and single bacterial species isolated from the community enrichments. Each isolated bacterium is highly abundant in the phycosphere-attached fraction of the enriched community, suggesting they could play a role in algal population dynamics. To test the hypothesis that these isolates may shape these algal-bacterial symbioses, we quantified the exchanges of C and N between the bacteria and the algal host using nanoscale secondary ion mass spectrometry (NanoSIMS). Indeed, phycosphere attachment of bacteria lead to a greater incorporation of fixed algal $^{13}CO_2$ products over unattached bacteria. While carbon transfer from algae to bacteria was confirmed, algal utilization of microbial-derived nitrogen via metabolite and/or vitamin release remains ambiguous. Algal health (quantified here as C fixation) is significantly affected by individual bacterial species, and the precise metabolites responsible for these observations are currently being examined. By combining tools such microbial community analysis with advanced isotopic imaging, we are generating a sensitive picture of how these interactions can be exploited to provide reliable and renewable fuels.

This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract DE-AC52- 07NA27344 and supported by the Genome Sciences Program of the Office of Biological and Environmental Research under the LLNL Biofuels SFA, FWP SCW1039. Additional funding was provided by the Laboratory Directed Research and Development Program at Sandia National Laboratories, which is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the US Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000.