Multitrophic considerations critical to understanding algal pond ecosystems

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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. https://bio-sfa.llnl.gov/

Considerable effort has been dedicated towards improving production of algal biomass for renewable bioenergy. There is a growing recognition that biotic factors – *i.e.*, interactions between algae and their microbiome, which includes microorganisms from all three kingdoms – can significantly affect algal productivity and offer tangible areas for improvement besides algal strain development and abiotic factors. In three highlighted studies of mass algal cultivation facilities described below, we explore several key biotic factors, namely functional attributes of bacteria associated with algal cultures and potential trophic interactions with competitors, predators and parasites. These findings highlight the importance of various aspects of algal pond ecology in algal culture outcomes and offer insights into unique ecology-driven cultivation improvements.

Algal mesocosms are dynamic systems in which the associated microbial communities change with concurrent changes in algal physiology and population density. To interrogate the functional capabilities of bacterial communities associated with algal cultures, six replicate outdoor mesocosms with *Microchloropsis salina* cultures were sampled for metagenomes at 3 weeks (Early) and 5 weeks (Late) after inoculation. Both time points included bacterial functions involved in lipid/nucleotide metabolism, as well as production of vitamins. Global functional gene differences indicated an increase in functional capability to degrade carbohydrates and to produce secondary metabolites, with a decrease in genes for signal transduction and quorum sensing. Binning the metagenomes resulted in 10 high-quality reconstructed genomes (MAGs)

with a diverse suite of functional potentials. Several MAG genomes code for functions that suggest they may acquire energy phototrophically (via bacteriochlorophyll and photosynthetic gene clusters). We continue to investigate how key microbiome functions, *i.e.* carbon capture/remineralization and secondary metabolite production, may aid algal photosynthesis and growth.

In order to further expand our knowledge on controls of pond dynamics, in a separate study, we examined the progression of both prokaryotic (16S rRNA) and eukaryotic (18S rRNA) community members in mesocosms seeded with either *M. salina* or *Phaeodactylum tricornutum* over a typical production timeline with multiple algal harvestings. Initially, the *P. tricornutum* ponds had variable taxa in each replicate pond but converged towards Alphaproteobacteria and eventually Flavobacteria over time. Conversely, the *M. salina* ponds were much less variable, having primarily Deltaproteobacteria throughout the experiment. Although each pond was initially seeded with only a single algal species, results show that several ponds experienced contamination from a neighboring algal species over the course of the experiment. In the *P. tricornutum* ponds, a pathogenic *Kordia* bacterial species bloom likely led to the premature crash in all replicate mesocosms. In the *M. salina* mesocosms, however, potentially pathogenic *Pseudobacteriovorax* species were abundant, yet no algal crash was observed. In all ponds, predatory ciliates were prevalent, with the most abundant families distinct to the host algal strain. These findings indicate that in algal mesocosms, multitrophic interactions are widespread and, though often overlooked, top-down controls may be key factors in pond dynamics.

In order to gain a better understanding of the role of a particularly prominent algal parasite in algal ponds, we conducted a time course sampling of algal microbiomes in *Haematococcus pluvialis* raceways frequently infected by chytrid fungi. The bacterial component of the pond microbiome displayed strong successional dynamics driven by changes both in culture age and algal physiology. Chytrid ecology was also associated with algal processes, with high pond infectivity only in cyst stages. Culture-based assays revealed a previously unrecognized non-infective chemoorganotrophic lifestyle. To extend these findings to the field, we developed a fungal group-specific qPCR method to assess abundances of chytrids and blastoclads. qPCR results confirmed that samples with active chytrid infections had high chytrid and/or blastoclad abundances, while also revealing that in some samples where no active chytrid infection was detected, abundances were still high – approximately 12-25% of those samples from infected ponds. This validates that the noninfective chytrid lifestyle is relevant to chytrid dynamics in algal ponds and suggests that abundant noninfective chytrids may make additional contributions to carbon and nutrient cycling processes in algal systems.