

Multi-Component Characterizations of Algal Pond Ecosystems Reveal Algal Influence on Heterotroph Dynamics

Christopher S. Ward¹ (ward56@llnl.gov), Kunal Poorey², Carolyn L. Fisher², Laura T. Carney³, Xavier Mayali¹, Michael P. Thelen¹, **Todd W. Lane²**, **Rhona K. Stuart¹**

¹Physical and Life Sciences, Lawrence Livermore National Laboratory, Livermore, CA;

²Department of Systems Biology, Sandia National Laboratories, Livermore, CA;

³Agricultural Science and Molecular Ecology, Heliae Development LLC, Gilbert AZ

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

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 processes.

Algal biomass production is a promising renewable energy source and mass algal cultures in open ponds are currently grown worldwide. Considerable research effort has gone towards understanding abiotic drivers of algal productivity; nevertheless, unpredictable biomass yields continue to contribute to high production costs above the target levels needed to achieve economic feasibility. Recently, there has been a growing appreciation that the algal pond microbiome, e.g. bacterial heterotrophs and pathogens, microeukaryotic competitors, may be vital in determining outcomes of productivity and other relevant pond metrics. Thus, understanding the complex environmental roles of the algal microbiome is critical for advancing algal biofuel production. Here, we present findings from several ongoing studies in which microbiome characterizations have revealed compelling features of algal pond ecology.

Time course sampling of algal microbiomes was conducted on open ponds and raceways of *Microchloropsis salina* CCMP 1776 in AZ Sonoran Desert and TX Gulf Coast and *Chlorella sorokiniana* DOE 1412 in northern AZ. The compositions of bacterial communities associated with each microalgal culture were distinct, yet we identified several taxa held in common constituting a core algal microbiome present in all cultivation trials despite differences in, culture media, and geographical regions. The *Microchloropsis* associated microbiomes from these two sites were compared to the TARA Oceans survey to examine whether these algal-bacterial interactions also occur in natural ecosystems. Further, temporally-resolved analyses showed that progressions in bacterial community composition over culture duration were a consistent characteristic of microbiome development observed across cultivation trials.

In a separate study of *Haematococcus pluvialis* raceways frequently infected by chytrid fungi, we undertook a multi-component characterization of the algal pond ecosystem (i.e., bacterial community, chytrid infection, algal growth and exudate composition) by combining field and lab analyses. Due to the distinct growth stages of *H. pluvialis* (i.e., flagellate, palmella, aplanospore), we were able to differentiate between the influences of algal physiological state and culture age on bacterial community composition. Indeed, the pond microbiome displayed successional dynamics significantly explained by changes both in culture age and algal physiology. Similar to the *M. salina* and *C. sorokiniana* pond microbiomes, these results suggest environmental filtering of the bacterial community by phycospheric changes in carbon composition and quantity with algal culture status (i.e., age and growth stage). To further explore this, NMR-based metabolite analyses were conducted to determine differences in exudate composition between algal growth stages. Chytrid ecology was also associated with algal processes, with high pond infectivity only in cyst stages. Culture-based assays supported the dependence of chytrid infectivity on algal life stage, while also revealing a previously unknown non-infective chemoorganotrophic lifestyle regulated by dissolved organic carbon (DOC) availability. While the full significance of multiple chytrid trophic strategies in the pond setting is still unclear, it may relate to interplay with algal exudates and resource competition between heterotrophic bacteria and chytrids. The more holistic approach to studying the algal pond ecosystem described here revealed numerous ecological interactions that connect components primarily through carbon economy and flow. Together, the findings provide insight into critical ecosystem processes and dynamics that may ultimately contribute to algal health and productivity.

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.