

## **Exploring the functional aspects of microalgal interactions with associated microbes, predators and pathogens**

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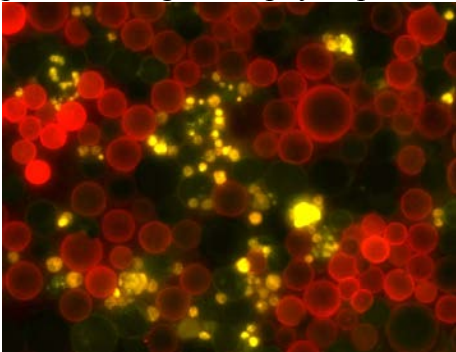
**Project Goals: The LLNL Biofuels SFA seeks to support robust and sustainable microalgae fuel production through a systems biology understanding of algal-bacterial interactions. We hypothesize that by understanding the factors that control cellular physiology and biogeochemical fluxes in and out of 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.**

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

Algae biomass production is a promising renewable energy source, and mass algal cultures in open ponds are currently grown worldwide. However, costs are above target levels needed to achieve economic feasibility, in large part due to biotic interactions that lead to unpredictable biomass yield in outdoor ponds. In open ponds, algae come in contact with numerous microbial ‘contaminants’, chiefly heterotrophic bacteria and predatory eukaryotes. Microalgal grazers and pathogenic fungi can cause rapid declines in algal populations in engineered mass algal cultures, as well as natural aquatic systems. However, we have a limited mechanistic understanding of how these organisms interact with microalgae and what roles bacterial members of the algal microbiome play in mitigating or enhancing these interactions. Understanding the ecological interactions that arise between algal cells and other microbial members in open systems is essential for managing algal health, and, ultimately, optimizing C flow into algal biomass.

Our previous studies of algal microbiomes indicate the bacterial community associated with *Nannochloropsis salina* is relatively stable in open cultivation ponds. To investigate the biochemical and metabolic roles that the microbiome provides for its host, we performed a temporal study in *N. salina* open systems to track profiles of microbial taxa and their metabolic pathways across three multi-generational algal cohorts. Shotgun sequencing of the time series of 26 samples yielded more than 300 million high quality sequence readings, assembling to ~230 million contigs, and 5.5 million ORFs (Open Reading Frames). By combining high-resolution taxonomic analysis, metabolic reconstruction and functional diversity assessment, we found evidence of complex relationships between microbiome composition and function that govern microbial community assembly during microalgal growth.

To better understand how top-down pressures shape algal population dynamics, we have developed simplified model systems (alga-rotifer and alga-chytrid) and are studying how their interactions influence the physiological and metabolic outcomes of both members, and how potentially beneficial phycosphere bacteria may influence these interactions. Results show that chytrids can interact with algae via metabolism of algal-derived organic matter and infection (Figure 1), though differences across chytrid strains and algal growth stages suggest that variable expression and sensing of biochemical factors may be responsible for differential outcomes. Further, grazing assays suggest that presence of certain bacteria can reduce the interaction of the rotifer grazer, *B. plicatilis*, with *N. salina*. Ongoing work will focus on biochemical and multi-omic approaches to examine the mechanistic underpinnings of metabolic and trophic interactions between predators, algae and phycosphere bacteria.



**Figure 1** Infection of algal culture in red cyst stage by *Paraphysoderma* 10 days after inoculation. Red spots: Algal cells (autofluorescence of chlorophyll), yellow spots: fungal sporangia stained with Nile Red

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