Characterizing algicidal bacteria and their mechanisms in antagonistic algal-bacterial interactions

Megan M. Morris (morris81@llnl.gov)[1], Jeffrey Kimbrel[1], **Rhona Stuart**[1], **Xavier Mayali**[1]

[1]Physical and Life Sciences, Lawrence Livermore National Laboratory, Livermore, CA, USA

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 multitrophic biological communities, to characterize the microscale impacts of single cells on system scale processes.

Heterotrophic bacteria play critical biogeochemical roles in surface aquatic ecosystems where they interact with photosynthetic microalgae via remineralization of algal-derived organic matter. The mutualistic nutrient exchange between algae and associated bacteria can shift towards commensalism, for example when opportunistic bacteria directly exploit algal resources, attacking and lysing algae and benefitting from lysed cellular nutrients. There are a multitude of factors that contribute to the strategy, mechanism, and activity of antagonistic bacteria; however, these are not fully understood and may vary widely depending on taxonomic identity of each partner and the ecological context in which the two partners interact. It is critical that we better understand algal-bacterial antagonism, as bacterial attack on algal populations may have drastic implications for nutrient flux in both natural ecosystems and engineered biofuel aquacultures. Using genome-resolved metagenomics, amplicon sequencing, advanced microscopy, and growth assays, we aimed to characterize the identity, genomic capacity, and mechanism of a novel bacterium with algicidal effects on the model photosynthetic diatom *Phaeodactylum tricornutum*.

The putative algicidal bacterium of this investigation is an unculturable bacterial species growing within a laboratory enrichment of a reduced-diversity bacterial community co-cultured with *P. tricornutum*. We sought to first quantify the effect of the algicidal community on the growth of *P. tricornutum*, and second to identify the algicidal bacterium within this community. In repeated, independent laboratory experiments we showed that the algicidal bacterium attacks *P. tricornutum* during the lag and early exponential growth phases, crashing the diatom population and preventing it from reaching high cell abundances in late log and stationary growth phases. The rate and magnitude of the bacterially-induced diatom population decrease were exacerbated by abiotic stress. We showed that increased temperatures were correlated with a more rapid and irreversible decline in *P. tricornutum*. To identify the bacterial species within the community

enrichment, we used 16S rRNA gene amplicon sequencing. Specifically, we sub-sampled the original bacterial community, and sub-enrichments which varied in their algicidal capacity – high, moderate, to no algicidal effect on *P. tricornutum*. We conducted a repeated time-series sampling of the bacterial community in conjunction with growth of *P. tricornutum* from inoculation (day 0) to mid-stationary phase (day 7). Bacterial sub-enrichments which varied in their algicidal phenotype were distinct in community structure and diversity, and we identified a taxon unique to the sub-enrichments with high algicidal capacity against *P. tricornutum*. Specifically, this taxon, an unclassified Alphaproteobacterium, reached high relative abundances in conjunction with the decline of *P. tricornutum*, comprising of up to 60 % relative abundance within the bacterial community. This suggests that the algicidal Alphaproteobacterium benefits in some capacity from the lysis of *P. tricornutum*. However, the exact strategy and mechanism of the algicidal activity by the unclassified Alphaproteobacterium are still unclear and will be the focus of follow-up experiments. Currently, we are describing the genomic capabilities and metabolic potential of the algicidal Alphaproteobacterium through a metagenome-assembled-genome (MAG) of this organism, as the recovered genome may elucidate the genetic underpinnings of the algicidal phenotype. In addition to characterizing a novel algicidal bacterium, this investigation more broadly contributes to our understanding of the spectrum of algal-bacterial interactions, and this has implications for both environmental and applied sciences.

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