

Title: A novel algicidal bacterium threatens diatom productivity and incorporates algal-derived carbon and nitrogen

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Project Goals: Algal and plant systems have the unrivaled advantage of converting solar energy and CO₂ into useful organic molecules. Their growth and efficiency are largely shaped by the microbial communities in and around them. The μ Biospheres SFA seeks to understand phototroph-heterotroph interactions that shape productivity, robustness, the balance of resource fluxes, and the functionality of the surrounding microbiome. We hypothesize that different microbial associates not only have differential effects on host productivity but can change an entire system's resource economy. Our approach encompasses single cell analyses, quantitative isotope tracing of elemental exchanges, 'omics measurements, and multi-scale modeling to characterize microscale impacts on system-scale processes. We aim to uncover cross-cutting principles that regulate these interactions and their resource allocation consequences to develop a general predictive framework for system-level impacts of microbial partnerships.

Abstract Text:

Photosynthetic algae, including phytoplankton, are globally significant CO₂ sinks and have high biotechnological potential for renewable biofuels and sustainable carbon sequestration. The heterotrophic bacteria that associate with microalgae in aquatic environments mediate biogeochemical cycling of algal-derived carbon and nitrogen, affecting the flow of nutrients to surrounding microbial cohorts and higher trophic levels. Through complex interactions and exchanges of secondary metabolites, bacteria can also influence algal productivity, either positively via mutualism or negatively via antagonism. Antagonistic algicidal bacteria lyse microalgal cells, diminishing populations and compromising or halting algal productivity. In the process, algal-sequestered nutrients are released that stimulate the metabolism of the surrounding microbial community, interfering with the system resource pool and economy. Catastrophic crashes within susceptible microalgal biofuel ponds are fairly common, and while these may be

attributed to biological entities such as algicidal bacteria, the causative agent is rarely investigated.

Here, we document a novel algicidal bacterium that lyses the biofuel-relevant diatom *Phaeodactylum tricornutum*, crashing laboratory cultures in days. While this novel bacterium is unculturable using traditional microbiological laboratory techniques, we use unique resources and molecular techniques to characterize the identity, strategy, and mechanism of this bacterium. Based on phylogeny of both a partial sequence obtained from amplicon sequencing and assembled 16S contig from a metagenome-assembled-genome (MAG), this is the first documented algicidal bacterium belonging to the taxonomic order Rickettsiales, which we name as a Rickettsia-like diatom killer, or “RLDK”. Through super-resolution confocal microscopy and fluorescence in situ hybridizations using custom probes, we identified RLDK within a laboratory enrichment of a mixed community. Unlike other Rickettsiales pathogens, RLDK appears to have a free-living life stage and is capable of survival outside of host cells; however, most RLDK cells were found associated with the diatom cells, especially just prior to algal death. Metabolic predictions from the MAG also confirmed RLDK’s genomic capacity for aggregation and attachment to host cells using pili. Further, the RLDK genome encodes a full type IV secretion system that may transport virulence factors and effector molecules extracellularly. In conjunction with peptidoglycanase-encoding genes, we hypothesize that RLDK’s mechanism of attack is a series of events where bacterial cells attach to *P. tricornutum* and secretes host cell-degrading peptidoglycanases.

RLDK is unable to survive in pure culture free of the diatom host or other bacteria. Additionally, this bacterium has a reduced genome with numerous auxotrophies. Based on these two pieces of evidence, we hypothesized that RLDK is likely dependent on other entities for its growth and metabolism. To test this, we conducted a NanoSIP experiment and found that diatom-attached bacteria (RLDK) show enrichment of both isotopically labeled ^{13}C and ^{15}N , neither of which RLDK can directly uptake or metabolize based on predictions from a genome-scale metabolic model. These results suggest that RLDK incorporates algal-derived carbon and nitrogen. Genome and metabolic model predictions will be tested using metatranscriptomes of both the bacterial parasite RLDK and algal host *P. tricornutum*. These data contribute to our understanding of poorly characterized algicidal bacteria in the environment and more broad understanding of algal-bacterial interactions. This research has implications for applied sciences, as our insights into the strategy and mechanism of algicidal bacterial activity may be leveraged to potentially prevent future catastrophic algal biofuel crash events.

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