

Title: Siderophore-Producing Phycosphere Bacteria Alleviate Iron Limitation Stress in *Phaeodactylum tricornutum*

Authors: Coffey NR^{1*} (coffeyni@oregonstate.edu), Rodriguez MCA¹, Rolison KA², Mayali X², Chu RK³, Kew WR³, Kimbrel JA², Boiteau, RM^{1,3}, Stuart RK²

Institutions: ¹College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, OR; ²Lawrence Livermore National Laboratory, Livermore, CA; ³Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA.

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

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: *Phaeodactylum tricornutum* (Pt) is a saltwater, low iron (Fe)-tolerant diatom ideal for use in studies probing the regulation of micronutrient availability by algae and their microbiome. While Pt's physiological response to Fe limitation has been fairly well-studied, its interactions with microbial partners is not well constrained. Microbial symbionts in Pt's phycosphere (a microscale zone of influence at/near the diatom's surface) may employ mutualistic strategies to enhance the growth of both the bacteria and the algae, possibly via the solubilization and subsequent bioavailability increase of micronutrient Fe. Through genome mining of bacteria isolated from Pt's phycosphere, three heterotrophic strains were identified with putative biosynthesis gene clusters for siderophores (small molecules that are secreted in response to Fe deficiency to solubilize and bind Fe to facilitate uptake). Initial results from co-culture experiments reveal enhanced growth of Pt in co-culture with each of these strains under low-Fe conditions relative to an axenic Pt control. Mass spectrometry based metallomic analyses of spent media reveal the production of metal chelators at low Fe concentrations within Pt-microbial cocultures but not in the axenic control. Notably, in response to Fe limitation stress, a novel siderophore was secreted by a Pt co-culture with bacterial isolate *Stappia* sp., a member of the Rhodobacteriaceae commonly found associated with microalgae in nature. This siderophore is believed to be produced via a nonribosomal peptide synthetase pathway by *Stappia* sp.. These results suggest that microbial partners may facilitate algal uptake of Fe when it may otherwise

limit algal growth and production. Since siderophore-bound Fe can only be utilized by microbes that can actively take up those specific molecules, this strategy may also play a broader role in managing Fe bioavailability by promoting the growth of certain taxa within a complex community. Metabolomic studies such as these are a promising avenue for understanding how host-microbe interactions manage resource allocation and contribute to algal biofuel pond community composition and resiliency.

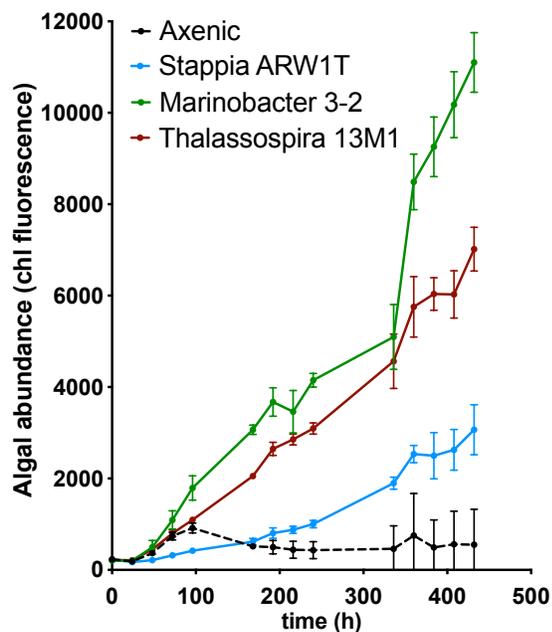


Figure 1: *P. tricornutum* growth response in 0.01 μM Fe in coculture with various single phycosphere taxa (*Stappia*, *Marinobacter* or *Thalassospira*) or axenic. Error bars represent standard deviation between 4 biological replicates.

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