Title: System-Level Analysis of Metabolism in a Novel Algicidal Bacterium

Authors: Helena van Tol,[1] Genevieve Parkey[2], Megan Morris[1], Jeffrey Kimbrel[1], **Rhona Stuart**[1], Patrik D'haeseleer[1], Xavier Mayali[1], **Ali Navid[1]*** (navid1@llnl.gov)

Institutions: [1]Physics & Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; [2]Georgetown University, Washington, DC

Website: https://bio-sfa.llnl.gov/

Project Goals: Algal and plant systems have the unrivaled advantage of converting solar energy and CO_2 into useful organic molecules. Their growth and efficiency are largely shaped by the microbial communities in and around them. The µBiospheres 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.

Planktonic algae play an outsized role in regulating the dynamics of earth's ecosystem. Their activity affects the global oxygen supply, the food chain, and climate. Due to their ecological importance, as well as the industrial interest in using algal biomass for production of renewable biofuels, understanding the factors that control algal population dynamics are imperative for devising strategies to develop renewable fuels and lessen the harmful effects of climate change. Interactions of algae with bacteria are a major factor in the fate of algal populations.

As part of our system-level examination of microbial interactions with bioenergy-relevant phototrophs, we found that a previously uncharacterized Rickettsia-like diatom killing (RLDK) bacterium was crashing diatom *Phaeodactylum tricornutum* cultures while increasing in relative abundance in conjunction with algal decline. Unfortunately, RLDK cannot be isolated in pure culture. Additionally, our experimental and bioinformatic analyses have indicated that its mechanism of killing is different from other well studied algicidal organisms like *Kordia algicida*.

In cases where direct lab examination of an organism is infeasible, in silico analyses serve as a powerful alternative method of study. So, to examine the novel algicidal mechanism of RLDK, particularly the role that its metabolic characteristics might play in this transient behavior, we characterized its metabolism by developing a genome-scale model of its metabolism using a high-quality, near-complete metagenome-assembled genome (MAG).

We used several steps to generate a relevant genome-scale model of this novel bacterium. We initiated the model development process by using the new apps developed by members of our team for the DOE KBase platform to import and merge annotations of the RLDK MAG from a number of different sources. This because we have previously shown that combining annotations from multiple sources will provide us with a more complete annotation and subsequently metabolic network reconstruction[1]. We then used the KBase to generate a draft genome-scale model. TranSyt (a KBase app used to annotate and add transport reactions)[2] was used to identify new gene-protein-reaction associations and many new transport reactions were added.

Despite constraint-based system-level analyses showing that RLDK's algicidal activity does not stem from a need to scavenge any specific organic compound from algal remains, preliminary simulations and bioinformatic analyses point to several interesting metabolic characteristics. For example: 1) examination of the RLDK genome shows that it contains a Type IV secretion system, as well as a surprising number of peptidoglycan degrading enzymes which could point to the means by which it attacks the algae; 2) flux balance analysis of RLDK metabolism indicates that it uses a non-orthodox pathway for production of thiamine phosphates which results in production and export 4-hydroxybenzyl alcohol, a reduced form 4-hydroxybenzoate, a metabolite that our metabolomic studies indicate could have a significant role in algal-bacterial interactions; 3) RLDK metabolism of some compounds can result in production of superoxides, a practice of heterotrophic bacteria when they need to acquire trace metals or to prevent viral infections[3]. This suggests that the impetus for RLDK's algicidal activity may be collection of inorganic nutrients instead of organic resources. We are currently analyzing proteomics and transcriptomic data to ascertain RLDK metabolic changes that facilitate its diatom killing activity. This research has provided a suite of new hypotheses to guide our experiments, illustrating the value of systems-level in silico analysis to support experimental work, and uncovered new potential resources and metabolites which may govern our algal-bacterial interactions.

References/Publications

1 Griesemer, M., Kimbrel, J. A., Zhou, C. E., Navid, A. & D'haeseleer, P. Combining multiple functional annotation tools increases coverage of metabolic annotation. *BMC genomics* **19**, 948 (2018).

2 Lagoa, D. *et al.* TranSyT, the Transport Systems Tracker. *bioRxiv*, 2021.2004.2029.441738, doi:10.1101/2021.04.29.441738 (2021).

3 Diaz Julia, M. *et al.* Widespread Production of Extracellular Superoxide by Heterotrophic Bacteria. *Science* **340**, 1223-1226, doi:10.1126/science.1237331 (2013).

Funding Statement: *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 μBiospheres SFA, FWP SCW1039.*