System-level analyses of beneficial interactions in an algal-bacterial co-culture

Ali Navid[1] (navid1@llnl.gov), Miriam Windler[2], Alfred Spormann[2], Patrik D'haeseleer[1], Mary Lipton[3], Sam Purvine[3], **Xavier Mayali**[1] and **Rhona Stuart**[1]

[1]Physical & Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; [2]Department of Civil and Environmental Engineering, Stanford University, Stanford, CA; [3]Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA

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

As part of our SFA, we have generated global expression data for algal-bacterial co-cultures, for which we are interested in the regulation of their metabolic interactions. We apply metabolic modeling approaches to this proteogenomic data to both constrain our models, interpret our results, and inform follow-up experiments. One illustration of the utility of this approach comes from our investigation of the interaction between the model green algae *Chlamydomonas reinhardtii* and an Actinobacterium, *Arthrobacter sp.* strain P2b. Our experimental results demonstrated increased algal biomass in the presence of P2b cells as well as cell-free P2b spent medium. Moreover, the results showed that the active compounds in the spent medium were heat resistant and smaller than 3 kDa, suggesting that small molecules could be involved. We next examined changes in algal global protein expression in response to both P2b co-culturing and P2b spent medium, to try and determine which molecules may be responsible for the changes in algal physiology observed.

To gain a more systems-level understanding, we analyzed the proteomic expression data using a human-curated genome-scale computational model of metabolism in *C. reinhardtii*[1] with our in-house developed GX-FBA[2] method of constraining flux balance analysis models with transcriptomic and proteomic data. The GX-FBA analyses identified two processes that were consistently differentially regulated between axenic cultures of *C. reinhardtii* and those when it was either grown in a co-culture with P2b or in medium containing P2b spent medium. In all

cases we found that compared to the axenic culture 1) the pathways for production of sulfur containing amino acids (i.e. cysteine and methionine) and 2) the pathway for production of vitamin D3 were upregulated. We hypothesize that the first metabolic response may be due to cysteine being a precursor for production of glutathione, a potent antioxidant. Glutathione may be needed to offset the increased production of harmful reactive oxidative species (ROS) that are produced during photosynthesis under high algal biomass.

We followed up these results by directly testing the addition of vitamin D3 to *C. reinhardtii* and *Chlamydomonas*-P2b co-cultures, grown under two different media. Co-cultures grown in P49 media (with yeast extract and tryptone), which allows P2b growth, exhibited a 100% growth increase when 1 micromolar vitamin D3 was added. Co-cultures grown in Bold's medium, with no organic C and N sources, where P2b cannot grow, exhibited a more modest but still significant 38% increase in algal growth. Vitamin D3 additions to axenic *C. reinhardtii* did not exhibit growth increases, suggesting strain P2b needs to metabolize vitamin D3 into a different component in order to exhibit mutualistic effects. Together, these results demonstrate the utility of applying metabolic models to direct follow-up experiments, and indicate that vitamin D3 may play an important role in *C. reinhardtii* -bacterial interactions.

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2 Navid, A. & Almaas, E. Genome-level transcription data of Yersinia pestis analyzed with a New metabolic constraint-based approach. *BMC Syst Biol* **6**, 150, doi:10.1186/1752-0509-6-150 (2012).