In silico system-level analysis of interactions in algal-bacterial co-cultures

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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 processes.

Microorganisms grow and adapt in a dynamic and diverse set of environments, all while interacting with other species. In fact, many organisms like algae owe their versatile set of metabolic capabilities to the community interactions they have with others [1]. In depth knowledge about causes and outcomes of these interactions are critical for assessing the robustness of natural and engineered microbial communities to various types of genetic, physical and population perturbations. However, characterizing the molecular mechanisms and regulatory processes that govern these interactions is challenging. Computational modeling that is informed by system-level experimental data is the key tool for examining the mechanisms and regulatory processes that govern these interactions and can be used to circumvent problems associated with experimental shortcomings. Here we report on our use of such modeling to characterize an algal-bacterial interaction. As part of our SFA, we are investigating the metabolic interactions of a number of microalgae with their bacterial symbionts, including the model green algae *Chlamydomonas reinhardtii* with an actinobacterium, *Arthrobacter sp.* P2b (P2b). The aim of these analyses is to understand the effect of these algal-bacterial interactions on algal biomass production. We compared *C. reinhardtii* and P2b co-culture growth to *C. reinhardtii* grown on its own (axenically). We observed that the algal biomass production in co-culture with P2b is strongly enhanced, suggesting a beneficial or commensalistic interaction between the two species. Our results show that the chlorophyll content of the algae is up to 3 times greater in the co-culture. The higher algal biomass in the co-culture with P2b is due to both higher cell densities and larger *C. reinhardtii* cells. In addition, we determined that the cell-free spent media from P2b alone

promotes biomass growth. In order to gain more knowledge about the metabotypes of the bacterium, we carried out growth experiments in phenotype microarray BIOLOG plates containing minimal media plus different carbon and nitrogen sources. We found that P2b is capable of growth on about one-third of the 190 substrates tested.
To further characterize P2b's physiology, we sequenced the genome (JGI CSP#1939), and combined the results from several different annotation tools, namely: RAST, KEGG, EFICAz, and TransportDB's transporter automatic annotation pipeline to gain a more complete functional annotation. The latter analysis resulted in identification of 557 transporter-associated genes; more than half of which were assigned to a specific substrate. Our enhanced annotation of P2b genome finds a number of interesting pathway for production of key secondary metabolites, including those for production of phytohormones that are known to improve algal growth[2].
We used DOE's Kbase (https://kbase.us) platform to generate a draft genome-scale model (GSM) of P2b's metabolism. We have begun the process of curating the draft model using our improved annotation results as well as data from our P2b BIOLOG analyses. We ultimately intend to pair the curated P2b model with a published GSM of *C. reinhardtii* [3] using Dynamic Flux Balance Analysis (dFBA) [4]. DFBA would allow us to simulate the interaction between the two organisms and to explore the influence of the growth-promoting bacterium on algal biomass production, and overall system robustness to genetic and environmental perturbations. Concurrent to curating the P2b GSM, we have been working on augmenting the DFBA method to better simulate unique biochemical environment of algal phycosphere (*i.e.*, local concentrations and relatively large flux/substrate concentration values that could necessitate varying simulation time steps). Overall, the results of our experimental and in silico analyses suggest that: a) interaction between *C. reinhardtii* and P2b is not specific to this unique pairing, i.e, P2b produces the effector compound regardless of presence or absence of algae, b) the effector molecule might be a phytohormone, c) the concentration of the effector compound is low so proximity to algae would increase the efficacy of the interactions. We are currently examining the last point using our augmented DFBA models.

References

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