

Cross-Talk Through Microbial Interaction Coordinates Microbial Community Properties

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Project Goals: The PNNL FSFA goal is to identify the fundamental mechanisms by which microbial interactions and spatial organization impact rates and pathways of carbon and energy flow in microbial communities. The strategy involves the study of highly interactive and tractable model autotroph-heterotroph consortia whose member genome sequences have been defined. Our project leverages unique capabilities including multi-omics measurements, advanced functional imaging, taxonomic profiling and metabolic and regulatory network modeling to elucidate underlying reaction mechanisms within complex microbial communities. Our research plan supports DOE goals to achieve a predictive understanding of microbially-mediated carbon and energy transformation.

The means by which individual microorganisms affect each other's gene expression and coordinate metabolism in communities are largely unknown. To explore this, we interrogated specific interactions in a photoautotroph- heterotroph consortium to infer mechanisms that mediate metabolic coupling and acclimation to microbial partnerships centered on exchanges of reduced carbon and nitrogen. We constructed a binary consortium from a cyanobacterium, *Thermosynechococcus elongatus* BP-1 and an obligate aerobic heterotroph, *Meiothermus ruber* Strain A, that relies upon cyanobacterial organic carbon, O₂ and reduced nitrogen. We sought to identify the details of the interactions that supported growth of *M. ruber* and asked whether there were associated costs or benefits to the primary-producer, *T. elongatus*. We used species-resolved transcriptomic analyses in combination with growth and photosynthesis kinetics to infer species interactions and the environmental context under which they occur. In addition, we evaluated the transcriptional and physiological responses to partnerships across tightly controlled, nutrient-replete steady-states maintained via discrete energy inputs (incident irradiance) and

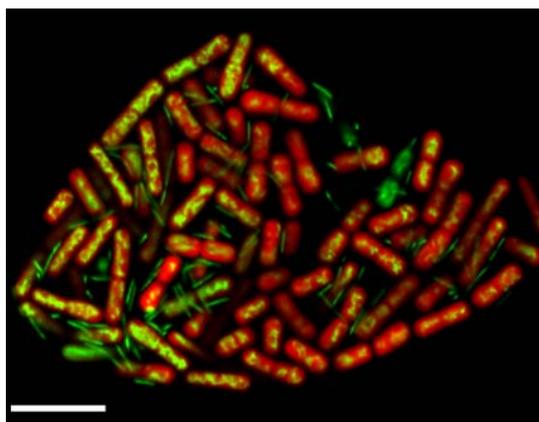


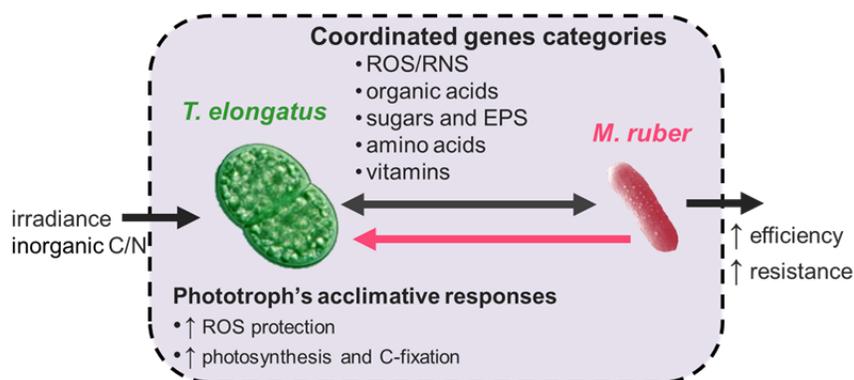
Figure 1. The *T. elongates* and *M. ruber* consortium was constructed to specifically test how microbes coordinate their metabolisms when exchanges of reduced carbon and nitrogen are essential for co-existence. We found that the cyanobacterium responded to its heterotrophic partner by altering transcriptional events and that the energy efficiency for biomass production is greater in the binary consortium as compared to the *T. elongatus* axenic control. The scale bar marks a 30 μ m distance.

nutrient-replete steady-states maintained via discrete energy inputs (incident irradiance) and

environmental stress imposed by elevated dissolved O₂ tensions. The guiding hypothesis for our work, to-date, is that that exchanged resources (i.e., reduced-C, -N, vitamins and O₂) act as the molecular singles that coordinated gene expression between the cyanobacterial producer and its heterotrophic partner.

We observed coordinated transcriptional responses from both species and used these to infer specific interactions resulting from the synthesis and exchange of resources, including amino acids, sugars and organic acids and other organic carbon sources. For example, the cyanobacterium responded to heterotrophic partnership by altering expression of genes involved with photosynthesis, carbon uptake, vitamin synthesis and scavenging of reactive oxygen species; the latter likely providing community-wide protection from oxidative stress. Heterotrophic partnership increased the efficiency of biomass production and resistance to stress induced by high levels of dissolved O₂ as compared to axenic cyanobacterial controls. Ongoing efforts to understand how these members might regulate each other's function are focused on the development of community-scale gene and metabolite association networks which can lead to the identification of interspecies, metabolic and regulatory coordination. From these networks, we are also building predictive genome-scale metabolic reaction networks to model specific exchanges of metabolites that may coordinate community-level behavior. By using the KBase Platform, we plan to predict specific interactions based on a novel network building procedure that integrates coordinated meta-transcriptional data directly into a interspecies network, the results of which are compared with conventional integration of data derived for individual species models (*see companion poster; Hyun-Seob Song et al. 2016*).

The resulting, genome-level discoveries and community metabolic models presented here provide a benchmark, systems-level foundation to infer specific interactions occurring between a unicellular cyanobacterium and its heterotrophic partner driven by direct metabolite exchange. Our findings, to date, support our hypothesis that microorganisms respond to partnership at the transcriptional level and are capable of acclimating to each other through indirect, interspecies regulation metabolic processes. We propose that some of the interactions, identified here, represent generalizable principles for autotroph-heterotroph community properties.



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