

Meta-Transcriptomic Network Analysis of Inter-Species Interactions in a Cyanobacterial-Methanotroph Coculture

Ryan McClure¹, Alexander Beliaev^{1*} (alex.beliaev@pnnl.gov), Pavlo Bohutskyi¹, Eric Hill¹, Marina Kalyuzhnaya², and **Jin Wang**³ (PI)

¹Biological Sciences Division, Pacific Northwest National Laboratory, Richland WA; ²San Diego State University, San Diego, CA; ³Auburn University, Auburn, AL;

Project Goals: In nature, microbial communities use highly efficient mechanisms to recover energy and capture carbon from both CH₄ and CO₂ through coupling of methane oxidation and oxygenic photosynthesis. However, harnessing the metabolic potential of phototroph-methanotroph consortia for biotechnology applications requires systems-level understanding of interspecies interactions and how these interactions affect the overall consortium dynamics and output. In this study we applied meta-transcriptomic network analysis to a model *Methylomicrobium alkaliphilum* 20Z - *Cyanobacterium stanieri* HL-69 coculture to identify the potential points of metabolic interactions between phototrophic and methanotroph bacteria. We expect that the knowledge gained from this work may be generally applicable to other cross-feeding consortia, and the tools developed can be adapted to study the interactions and dynamics of other multi-organism platforms.

Abstract: To reveal the specific points of interactions between *M. alkaliphilum* 20Z and *C. stanieri* HL-69, the coculture was grown under a range of environmental conditions that included variations in temperature, pH, dissolved O₂, nutrient concentrations and energy (*i.e.*, light) inputs under steady-state and batch growth regimes. Species-specific RNA sequencing was carried out to determine the transcriptomic response of each species. Global analysis of transcriptome patterns showed that batch conditions induced a broad range of response while bioreactor conditions were much more similar. Within batch conditions, temperature and nutrient type induced the largest changes in transcriptional response in *M. alkaliphilum* 20Z, while alterations in pH and dissolved O₂ induced response specifically in *C. stanieri* HL-69, respectively.

We next pooled all transcriptomic data into a gene co-expression network to examine the interspecies edges linking *M. alkaliphilum* 20Z and *C. stanieri* HL-69 subnetworks (Fig. 1). These points of transcriptional coordination can be used to identify which pathways and processes are likely linked between the species as well as to infer instances of cross-species interaction. Within the entire coculture network comprised of 20,000 total edges (links describing coordinated expression of two transcripts), 2,393 of them were found to be interspecies (linking a gene in *M. alkaliphilum* 20Z with one in *C. stanieri* HL-69). This subnetwork included 1,182 *M. alkaliphilum* 20Z genes and 1,022 *C. stanieri* HL-69 genes. Further examination of the cross-species subnetwork revealed that genes enriched in amino acid metabolism, energy metabolism, cell structure and transport occupied positions of high centrality indicating that these processes are likely to be critical for co-culture growth. Notably, the distribution of cross-species edges in the coculture network varied significantly as a function of cultivation regimes; we found that the number of cross-species edges was ~ 4-fold higher under steady-state conditions compared to batch cultures. This suggests that coordination between species is much higher under steady state vs. batch growth conditions. We attribute this discrepancy to the variation in specific growth phases of *M. alkaliphilum* 20Z *C. stanieri* HL-69 in batch cultures as the both populations in the coculture growing in steady state was

synchronized. Functional analysis of cross-species edges enriched in a specific function showed that carbohydrate metabolism of *M. alkaliphilum* 20Z was positively correlated with transport processes in *C. stanieri* HL-69 as were amino acid metabolism and translation, respectively. These experiments are among the first to build a co-expression network linking genes across species in a phototroph-methanotroph system and the knowledge gained will be critical not only in understanding interactions specific to these two functional guilds but also in building paradigms that describe fundamental coordination of metabolism and exchange of nutrients that are common to a large number of bacterial communities involved in the cycling of CH₄ and CO₂.

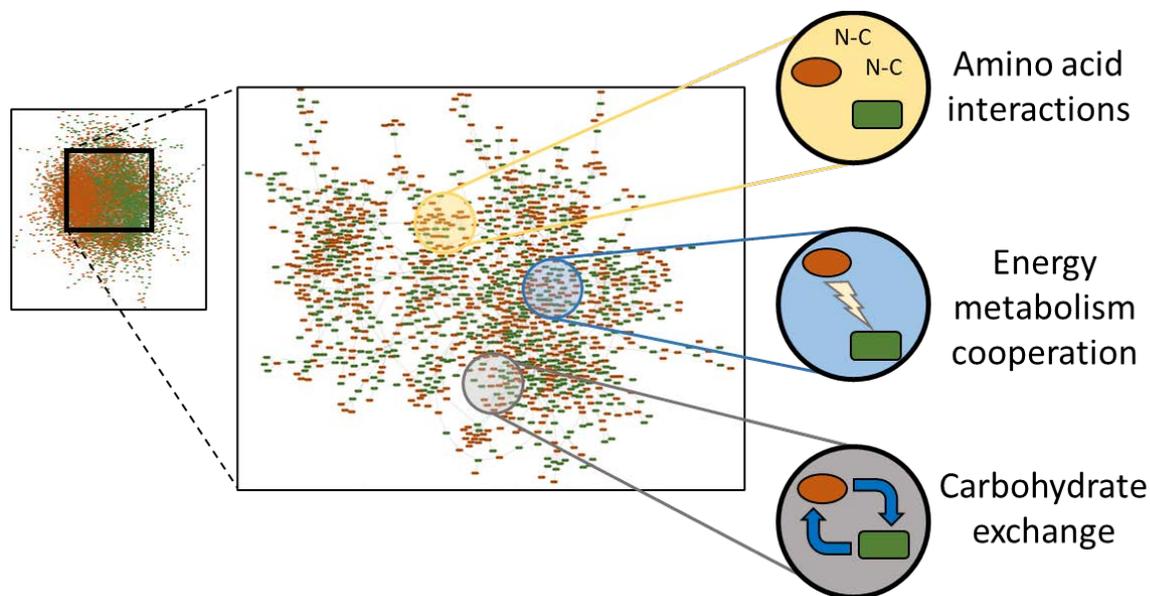


Figure 1. Meta-transcriptome network of *M. alkaliphilum* 20Z and *C. stanieri* HL-69 coculture. Green rectangles represent *C. stanieri* HL-69 genes and orange rectangles represent *M. alkaliphilum* 20Z genes. Lines indicate points of coordinated expression between genes with several pathways that are strongly linked (highlighted).

Funding statement: This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DE-SC0019181

