Predicting Autotroph – Heterotroph Metabolic Interactions from Genome Sequence

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Project Goals: The PNNL Foundational Scientific Focus Area, Biological Systems Interactions, investigates microbial interactions to understand how microbial communities work collectively to carry out complex biogeochemical processes. We seek to understand how the exchange of metabolites between organisms contributes to the stability of microbial communities. A longer-term goal is to develop predictive capabilities for the response of microbial communities to environmental change. A model system involving *Synechococcus* sp. PCC 7002 and *Shewanella* sp. W3-18-1 was selected to develop and test predictions of molecular exchange processes occurring during co-culture growth. To examine the molecular basis for this phototroph:heterotroph interaction we are applying comparative sequence analysis and experimental approaches to define and describe features in the genomes associated with their interactions and thereby identify functional traits important to microbial community structure. These investigations support lab-based investigations examining growth, physiology, metabolite exchange, and gene expression patterns in axenic and co-cultures.

The two model organisms chosen for our study, *Synechococcus* sp. PCC 7002 and *Shewanella* sp. W3-18-1**,** have been co-cultured without the supplement of nutrients supporting that their metabolisms can be coupled. *Synechococcus* sp. PCC 7002 is an oxygenic photoautotroph also capable of fermentative metabolism that produces different metabolic end products under variable environmental (i.e. light) conditions. *Shewanella* sp. W3-18-1 is a facultative anaerobe capable of utilizing a variety of carbon and energy sources.

Automated genome annotations of *Synechococcus* sp. PCC 7002 and *Shewanella* sp. W3-18-1 were curated to improve the specificity of functional assignments and to identify key knowledge gaps (e.g. missing steps in metabolic pathways, missing transport reactions) that require further investigation via computational & wet-lab methods. Gene models for both organisms have been adjusted using proteome data to validate protein starts and sub-cellular locations. Function predictions have included the use of experimental data from well-studied model genomes, such as *Synechocystis* sp. PCC 6803 (*Synechococcus*) and *S. oneidensis* MR-1 (*Shewanella*) as well as orthologs from phylogenetically related organisms. Pathway genome databases have been constructed for the *Synechococcus* and *Shewanella* pair. Regulatory interactions inferred in RegPrecise are included in the curation.

Comparative analysis of the *Synechococcus* sp. PCC 7002 and *Shewanella* sp. W3-18-1 genomes enables identification of the metabolic pathways that are unique to each organism. These pathways and their corresponding metabolites are a source of potentially exchanged nutrients that can contribute to the interspecies metabolic interactions. We also include pathways associated with compounds known to be present in autotrophic:heterotrophic consortia (i.e.

glycolate, propionate, lactate, acetate). Based on our pathway predictions we are cataloging potential interaction points for the *Synechococcus* sp. PCC 7002 and *Shewanella* sp. W3-18-1 pair. To establish the molecular connection between these cellular metabolic pathways and exchange nutrients, we generated a profile for the transport capabilities of these organisms. Inferred functional attributes were identified by sequence comparisons using Blast and reconciled with predictions from the Transporter Classification Database. To validate inferred transport functions, we examine the ligand binding preference for the ABC transporters. This family of transporters is ubiquitous in bacterial systems and provides import and export capability for a wide spectrum of ligands. A typical ABC importer generally consists of a periplasmic solute-binding protein, two integral membrane subunits, and two cytoplasmic ATPases. It is the solute binding proteins (SBPs) of the ABC transport complex that recognize and bind specific substrates in the cell wall and transfer them to the membrane subunits, and are therefore responsible for the uptake of ligands from the environment. We screened and characterized a set of transporter proteins from *Synechococcus* sp. PCC 7002 and *Shewanella* sp. W3-18-1 using a fluorescence thermal shift assay (FTS) and isothermal calorimetry. These functional screens, that included metals, small ions, mono- and oligosaccharides, peptides, amino acids, polyamines and vitamins, generated specific binding ligand assignments for approximately 60% of the purified and screened proteins. The experimental studies provide new functional information for these transport complexes and also validate many of the assignments derived from comparative analysis. These transport capabilities support the identification and characterization of metabolic and regulatory pathways for these organisms and provide a basis for experimental validation of the potential exchange nutrients that contribute to the interspecies metabolic interactions.

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