Metabolic Coupling and Carbon Flux Regulation in Phototroph-Heterotroph Associations

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Project Goals: The overarching goal of the PNNL Foundational SFA is to understand the collective energy, carbon, and nutrient processing in phototrophic microbial communities that contributes to their stability and efficient utilization of resources. As part of the FSFA cross-cutting science & technology theme, this research component elucidates pathways and regulatory mechanisms that govern metabolic exchange and interactions between organisms in phototrophic associations and contribute to their stability and efficient utilization of resources.

Scientific Concept: Secretion of low-molecular weight metabolites and biopolymers drives interactions between primary producers (autotrophs) and consumers (heterotrophs) and, at the organism level, is a function of environmental parameters such as light intensity, O_2 levels, and nutrient concentrations. Under photosynthetic conditions, O_2 is one of the key factors affecting carbon metabolism, growth, and survival of microorganisms. We hypothesize that photoautotroph-heterotroph associations have developed mechanisms to spatially and transiently decrease O_2 concentrations thereby lowering oxidative stress. Under dark conditions, the phototrophs ferment glycogen providing the heterotrophs with electron donor/carbon source to carry out anaerobic respiration which affects solubility of critical micronutrients such as Fe and Mn, thus making these elements more accessible for phototrophs. At a sub-cellular level, the interactions are mediated by multi-level regulatory networks that include redox-active proteins which sense environmental change through specific post-translational modifications and act as key control points to optimize metabolic efficiencies at both organism and community levels. To that end, we seek to identify regulatory proteins responsive to environmental change throughout the diel cycle, which includes redox-dependent disulfide bond formation and oxidative modifications to Met and Tyr (oxidative modifications are reversible through the action of repair enzymes). We hypothesize that light-responsive disulfide bond formation acts to control the functions of key proteins that regulate metabolic fluxes. Such adaptive regulation is suggested to diminish formation of reactive oxygen species (ROS), reduce protein oxidative damage, and enhance metabolic efficiencies. *In vivo* examples have been identified involving an inverse relationship between redox-dependent cystine formation and the abundance of site-specific oxidative modifications. As these post-translational modifications are reversible, they have the potential to rapidly reprogram metabolism through the control of specific branch points within metabolic pathways.

Our current line of research focuses on gaining an understanding of the pathways and regulatory mechanisms associated with the collective energy, carbon, and nutrient processing in axenic and mixed phototroph-heterotroph cultures. Systems under investigation include *Synechococcus* sp. PCC 7002 – *Shewanella putrefaciens* W3-18-1 co-culture, individual organisms (*e.g.*, *Synechococcus* spp., *Thermosynechococcus* sp., *Roseiflexus castenholzii*, *Chloroflexus* spp.), and consortia derived from, hot spring communities (e.g., *Thermosynechococcus* sp. N55-*Roseiflexus castenholzii*). This research also utilizes advanced controlled cultivation capabilities to enable investigation of redox-dependent control mechanisms.

Baseline growth parameters and the presence of regulated metabolic coupling between *Synechococcus* and *Shewanella* spp. have been identified, as both organisms successfully grow as a binary co-culture, but

not as separate monocultures, using either lactate or CO_2 as the sole source of carbon. During growth with lactate, O_2-dependent oxidation of lactate mediated by W3-18-1 produced sufficient amounts of CO_2 to maintain photosynthetic growth of 7002, which in turn, generates the necessary O_2 for growth of W3-18-1. These results establish that there is a tight metabolic coupling between the phototrophic and heterotrophic organisms using light as the only source of energy and lactate as the sole source of carbon. Growth of the co-culture under these conditions results in acetate accumulation by *Shewanella* W3-18-1 thus revealing an imbalance is caused by differences in growth rates where the O_2 consumption by the heterotroph exceeds the O_2 evolution rates by the cyanobacterium. When grown on CO_2 as the sole source of carbon, the metabolic coupling between 7002 and W3-18 is established through secretion of dissolved organic carbon compounds by the photoautotroph. NMR-based techniques identified formate, lactate, and acetate secreted by *Synechococcus* 7002 which may serve as the primary carbon and energy sources for *Shewanella* W3-18-1. Secretion of extracellular metabolites and/or biopolymers also appears to be common for some thermophilic cyanobacterial species, e.g., *Thermosynechococcus* sp. In hot spring phototrophic (HSP) mat communities major shifts in energy metabolism occur in response to increases in O_2 involving: *i*) alterations in metabolic exchange between community members through the release of acetate, and *ii*) diminished rates of carbon fixation due to photorespiration that may be associated with an uncoupling of RuBisCo and futile cycling involving glycolate production. These results indicate that enhanced metabolic efficiencies in communities of phototrophs and heterotrophs may arise, in part, due to increased efficiencies of carbon fixation as a result of reductions in photorespiration due to decrease in O_2 levels.

Additional cellular responses that impact community stability were found using chemical probes; they involve regulatory mechanisms that shift metabolic flux to minimize the oxidative damage to cellular proteins in a manner that will enhance metabolic efficiencies. Large decreases in the overall levels of protein oxidation are observed for cultures of 7002 grown in the presence of the W3-18-1, where the majority of oxidatively sensitive proteins in W3-18-1 are protected from oxidative modification in the co-culture. Reductions in oxidative stress are apparent despite substantially higher O_2 present in the co-culture (160% dissolved air saturation) in comparison to axenic cultures (44% dissolved air saturation) (both cultures are grown in caged photobioreactors using white light intensities of 240 μM photons m^{-2} s). These results support the hypothesis that opportunistic interactions between heterotrophic (*Shewanella*) and photosynthetic (*Synechococcus*) organisms permit metabolic coupling to enhance energy efficiencies and community stability. These measurements are consistent with prior indications that axenic isolates of the *Synechococcus* isolated from the HSP mats have a substantially enhanced sensitivity to light-induced oxidative stress in comparison to the natural mat community, indicating an importance of metabolic coupling between community members in the mat that promote stress resistance.

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