Anoxic Carbon and Nitrogen Metabolism in Photosynthetic Microbial Mats as Revealed by Metatranscriptomic and NanoSIMS Studies

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Photosynthetic microbial mats found in coastal environments are complex, stratified microbial communities that fix CO_2 during the day and ferment the fixed carbon under anoxic conditions at night, generating significant amounts of H_2 and organic acids. Fermentation of accumulated photosynthate may be required to provide energy for anoxic N_2 fixation. To understand C and N flux through these mats at a single cell and community level and to relate this flux to the observed rates of H_2 evolution and N_2 fixation, we have analyzed the upper 2 mm of cyanobacterial mats collected from Elkhorn Slough, CA, which have been shown to possess high rates of fermentation and N_2 fixation by metatranscriptomic and NanoSIMS studies.

We characterized the active microbial community from two mats samples collected under dark, anoxic conditions using metatranscriptomic sequencing to define the metabolic pathways for fermentation and N_2 fixation. Clone library and 454 pyrosequencing of the rRNA expressed by this community demonstrated that the active community was dominated by filamentous cyanobacteria including populations closely related to *Microcoelus chthonoplastes* and a novel diazotrophic cyanobacterial isolate, ESFC-1. Approximately 388,000 transcripts (average length ~390 bp) were obtained by sequencing amplified cDNA. Of these transcripts, ~199,000 transcripts (51%) were classified as protein coding sequences by comparison to the SEED database (bit score>40). Transcripts related to *Cyanobacteria* and *Chloroflexales* represent >70% of the total classified sequences in the transcriptome, suggesting that representatives of these phyla are active in the mats under anoxic conditions.

Metabolic reconstruction of the *Microcoleus chthonoplastes* genome identified a complete pathway for fermentation of glycogen to lactate, acetate, formate, ethanol and H_2. Fragment recruitment of metatranscriptomic reads indicated that all the genes in this fermentation pathway were expressed, consistent with organic acid and H_2 evolution measurements. A large number of metatranscriptomic reads affiliated with the *Chloroflexales* were annotated as acetate/lactate permeases and polyhydroalkonoate synthases, suggesting that the *Chloroflexales* may metabolize the organic acids produced by the cyanobacteria further and also synthesize carbon storage compounds to be utilized in photoheterotrophic growth. Assembly of the metatranscriptomics reads revealed that partial *nif* operons were reconstructed with the structural nitrogenase genes (*nifHDK*) for *Microcoleus,* ESCF-1 and an uncultivated member of the *Chloroflexales*, suggesting that these populations were responsible for the observed N_2 fixation activity.

These metatranscriptomic studies were complemented by NanoSIMS measurement of mat samples collected from Elkhorn Slough and incubated with isotopically labeled substrates. Incubation of mat samples with $^{13}HCO_3^-$ provided evidence for the fixation and storage of carbon by *Microcoleus* populations during the day and release of the stored carbon under anoxic conditions. Addition of ^{13}C-labeled acetate resulted in isotopic enrichment of the *Chloroflexi*, providing further evidence for the anoxic food web hypothesis generated by analysis of the mat metatranscriptome. Incubation of the mats with $^{15}N_2$ demonstrated that cyanobacterial populations related to ESFC-1 were highly active in N_2 fixation under dark anoxic conditions; however no evidence was found for incorporation of $^{15}N_2$ into *Microcoleus* and *Chloroflexi.*