

150. Diel Metagenomics and Metatranscriptomics of Elkhorn Slough Hypersaline Microbial Mat

Jackson Z. Lee¹§, Ulas Karaoz²* (ukaraoz@lbl.gov), Angela Detweiler¹, Craig Everroad¹, Leslie Prufert-Bebout¹, Rhona Stuart³, Mary Lipton⁴, Eoin L. Brodie², Peter Weber³, Brad Bebout¹, Jennifer Pett-Ridge³

¹INASA Ames Research Center, Mountain View, CA. ²Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA. ³Lawrence Livermore National Laboratory, Livermore, CA, ⁴Pacific Northwest National Laboratory, Richland, WA. §Current Affiliation: MIT Lincoln Laboratory, MA.

Project Goals: The LLNL Biofuel SFA investigates systems biology of complex microbial communities relevant to bioenergy production. To understand nutrient cycling and potential biofuel production in complex microbial communities we employ an integrated analysis of energy flow using multi-scale approaches including biogeochemical, stable isotope probing, metagenomic/transcriptomic, proteomic/metabolomic and computational analyses. Our ultimate goal is the development of multi-scale models that can predict ecological and biochemical relationships within multi-trophic microbial systems.

Microbial mats are amongst the most diverse microbial ecosystems on Earth, inhabiting some of the most inclement environments known, including hypersaline, dry, hot, cold, nutrient poor, and high UV environments. Photosynthetic microbial mats found in intertidal environments are stratified microbial communities with anoxic conditions at night, generating significant amounts of H₂ and organic acids. The high microbial diversity of microbial mats makes for a highly complex series of ecological interactions. To address this challenge, we are using a combination of metagenomics, metatranscriptomics, metaproteomics, iTags and culture-based simplified microbial mats to study biogeochemical cycling (H₂ production, N₂ fixation, and fermentation) in mats collected from Elkhorn Slough, Monterey Bay, California.

To understand the variation in gene expression associated with the daytime oxygenic phototrophic and nighttime fermentation regimes in hypersaline microbial mats, a contiguous mat piece was sampled at regular intervals over a 24-hour diel period. Additionally, to understand the impact of sulfate reduction on biohydrogen consumption, molybdate was added as an inhibitor to a parallel experiment. Four metagenome and 12 metatranscriptome Illumina HiSeq lanes were completed for samples collected from day / night, and control / molybdate experiments.

Our preliminary examination of gene expression in midday versus midnight samples (mapped using bowtie2 to reference genomes) has revealed several notable features, particularly relevant to the dominant mat-building cyanobacterium *Microcoleus chthonoplastes*.

M. chthonoplastes expresses several pathways for nitrogen scavenging, including nitrogen fixation. Reads mapped to *M. chthonoplastes* indicate expression of two starch storage and utilization pathways, a starch-trehalose-maltose-glucose pathway, and a UDP-glucose- cellulose-β-1,4 glucan-glucose pathway. The overall trend of gene expression was primarily light driven up-regulation followed by down-regulation in the dark; much of the remaining expression profile appears to be constitutive. Metaproteome analyses, conducted in collaboration with PNNL's Pan-Omics project (mapped using co-assembled metagenome), indicate upregulation of Chloroflexi-assigned proteins in the dark and upregulation Cyanobacteria-assigned proteins in the light

Co-assembly of quality-controlled reads from 4 metagenomes was performed using Ray Meta with progressively smaller K-mer sizes, with bins identified and filtered using principal component analysis of

coverages from all libraries and a %GC filter, followed by reassembly of the remaining co-assembly reads and binned reads. A total of 20 near-complete (>80%) and an additional 50 minor genomic bins have been identified. Despite having relatively similar abundance profiles in each metagenome, this binning approach was able to distinctly resolve bins from dominant taxa, as well as sulfate reducing bacteria that are critical to our understanding of molybdate inhibition effects. Bins generated from this iterative assembly process are being used for downstream mapping of transcriptomic reads as well as isolation efforts for Cyanobacteria- associated bacteria.

Publications

1. Lee, J.Z., L. Burow, D. Woebken, R.C. Everroad, M.D. Kubo, A. Spormann, P.K. Weber, J. Pett-Ridge, B.M. Bebout, T.M. Hoehler. (2014) Fermentation couples Chloroflexi and sulfate- reducing bacteria to Cyanobacteria in hypersaline microbial mats. *Frontiers in Microbial Physiology and Metabolism*. 5:61.

This research was supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under the LLNL Biofuels SFA, FWP SCW1039. Work at LLNL was performed under the auspices of the U.S. Department of Energy under Contract DE-AC52-07NA27344 and at Pacific Northwest National Lab supported by the OBER/GSP under the Pan-Omics project. Work at LBNL was performed under the auspices of the U.S. Department of Energy under Contract DE-AC02-05CH11231.