SYSTEMS-LEVEL INSIGHTS INTO ALTERNATE METHANE CYCLING MODES IN A FRESHWATER LAKE VIA COMMUNITY TRANSCRIPTOMICS, METABOLOMICS AND NANOSIMS ANALYSIS

M. G. Kalyuzhnaya[1], S. Yang[1], D. A. C. Beck[1],J.Bickford[1], M. C. Konopka, L. Chistoserdova[1] , V. J. Orphan[2], and **M. E. Lidstrom[1*]**

[1]University of Washington, Seattle, WA

[2]California Institute of Technology, Pasadena, CA

lidstrom@u.washington.edu

Project Goals: The goal of this project is to initiate a systems-level approach to studying natural communities involved in alternate modes of methane oxidation, such as aerobic versus anaerobic; and nitrate-dependent versus oxygen-dependent modes, in order to bridge gaps in understanding the specialized bacterial communities involved in these processes.

The project takes advantage of a large metagenomic dataset enriched in the DNA of the methanotrophic species employing different types of methane metabolism generated in collaboration with the Joint Genome Institute.

Our research was focused on the following three objectives:

Objective 1: Identify actively transcribed pathways. Next generation sequencing-based transcriptomic profiling (RNA-seq platform) was used to perform global characterization of C_1-metabolism in lake sediment, as well as community responses to stimulated environmental perturbation, such as low oxygen and additional nitrate**.** Reconstruction of the related C_1-metabolic function was performed. The results demonstrated that the sediment C_1- community is represented by microbial species adapted to low oxygen and low methane flux.

Objective 2: Identify physiologically active pathways. A highly comprehensive ion exchange solid phase extraction (SPE) liked with hydrophilic interaction liquid chromatography (HILIC-MS/MS) and liquid chromatography with a pentafluorophenylpropyl column (LCPFPP-MS/MS) was developed to understandcentral carbon metabolites involved in methane assimilation and adjacent pathways in complex natural mixtures. The recovery of 51 targeted metabolites from five compound classes (amino acids, carboxylic acids, sugar phosphates, nucleotides, and acyl-CoAs) was investigated. The following SPE procedures were employed: (a) mixed mode strong cation exchange, (b) mixed mode strong anion exchange, and (c) mixed mode weak anion exchange. We analyzed 32 of the targeted 51 metabolites using either HILIC-MS/MS or LCPFPP-MS/MS after SPE sediment samples cleanup and pre-concentration. The remaining 19 targeted metabolites were either at, or below, the detection limit. The current approach provides a good workflow for absolute quantification of intermediates in C_1-carbon metabolismin naturalmicrobial communities.

Objective 3: Identify activity of individual cells. As part of Objective 3, 10 individual active cells with unusual phylogeny were selected for whole genome amplification, sequencing, assembly, and functional characterization as a result of metabolic reconstruction. The result couples genomics to function, and therefore, has the potential to reveal newinsights into the physiological capabilities of yet uncultured members of natural microbial communities.

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