77. Systems Level Insights into Alternate Methane Cycling Modes Phase II: Deciphering Mechanistic Details of Interspecies Cooperation Through Manipulation of Model Microbial Communities

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Project Goals: (1) To develop model synthetic communities active in methane utilization through employing axenic cultures of methane-oxidizing bacteria, non- methanotrophic methylotrophs and non-methylotrophic heterotrophs, and to manipulate these communities by modifying methane and oxygen partial pressures followed by observations on growth and performance of the communities and behavior of each of the partners; (2) To identify candidate factors involved in interspecies cooperation through utilizing comparative transcriptomics and metabolomics; (3) To validate hypotheses via strain and community manipulation employing site-specific mutagenesis and/or introduction of synthetic functional modules to metabolically modify strains of interest and by substituting modified strain variants for wild type strains in manipulated microcosms. To Integrate data and use phenotypes and growth characteristics for developing and constraining single strain and community metabolic models.

In the first phase of this project, we investigated populations of microbes in a freshwater lake (Lake Washington) sediment active in methane oxidation at different oxygen tensions, using systems biology approaches such as microcosm manipulations, (meta)genomics, (meta)transcriptomics, (meta)metabolomics as well as pure culture manipulations. The major discoveries from this project include identification of the *Methylococcaceae* family microbes and specifically the *Methylobacter* species as key players in methane oxidation not only in aerobic but also in microaerobic conditions, and a rapid transfer of carbon originating from methane to non-methanotroph species, suggesting that metabolism of methane must involve a food chain rather than a single type of microbe (1). We have also uncovered one mechanism by which *bona fide* methanotrophs may support non-methanotroph communities, a novel type of methane metabolism involving fermentation (2). At the same time we have observed that communities active in methane metabolism are not random combinations of species, instead these are communities involving specific bacterial types, the most prominent being the *Methylotenera* species (3).

In this new phase of the project that commenced on September 1, 2013, we are addressing the nature of these proposed relationships among different physiological groups of microbes and the mechanisms underlying their specificity. In order to obtain mechanistic details into interspecies cooperation as part of the methane cycle, we are now using both top-down and bottom-up community manipulation approaches. In the first, we manipulate natural communities using methane as the only carbon source and monitor complex community deconvolution toward dramatically simplified, several-species communities. In the second, we use pure cultures of bacteria, all originating from Lake Washington sediment, all with sequenced genomes, to build and manipulate simple synthetic communities. At this meeting we will present data from the top-down approach demonstrating that, under methane pressure, the complex natural communities simplify rapidly, with *Methylobacter* species becoming one of the dominant species under two different oxygen regimens (referred to as ‘high’ and ‘low’). The communities, sampled at multiple time points using 16S rRNA gene pyrosequencing, revealed dynamic behavior with respect to species accompanying *Methylobacter*, which, while representing a significant portion of the total population at each given time, progressed through a series of population sweeps. The observed community dynamics differed between the ‘high’ and ‘low’ oxygen condition microcosms in terms of the dominant species composition.

Notably, the *Methylotenera* species that were the first responders to the methane stimulus in both conditions were replaced by the *Methylophilus* species in the 'high' oxygen conditions, followed by successions of various non-methylotrophic heterotroph species.

In contrast, the *Methylotenera* species persisted in the 'low' oxygen conditions. Moreover, in these conditions, we were able to observe dynamics between two different ecotypes of *Methylotenera*. Insights into the physiology of the major species forming these simple methane-utilizing communities were gained through metagenomic sequencing revealing differences in the genomic blueprints of the dominant species, including differences in major pathways for carbon and nitrogen metabolism. These data from the top-down approach suggest the most prominent models to employ in the bottom-up synthetic community experiments.

References

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