

New Insights into Methane-Oxidizing Communities in Lake Sediments through Microcosm Manipulation and Systems Biology Studies

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Project Goals: This project addresses the structure and function of microbial communities active in methane consumption, using lake sediment as a model. We utilize both top-down and bottom-up approaches toward this goal. The top-down approach involves manipulation of native sediment samples under specific environmental conditions, such as methane/oxygen/nitrogen species availability/relative concentrations. The bottom-up approach employs axenic cultures of model bacteria for constructing synthetic communities of different complexity, from very simple (two-species) to relatively complex (50-species). Through manipulations of these communities, combined with systems biology studies, we are striving to understand the molecular mechanisms that form a basis for specific species interactions in microbial oxidation of methane.

In the current phase of the project, as part of the bottom-up approach, we carried out microcosm incubations under two different oxygen tension regimens ('low' versus 'high'), in multiple replicates, over the course of 14 weeks, with microcosm cultures transferred with dilutions once every week. Samples of DNA and mRNA from week 4 to week 14 have been shotgun sequenced, with 4 replicates for each sampling point, in collaboration with the JGI. We are currently in the process of analyzing this extensive dataset that represents species active in methane oxidation, over time, as well as their respective activities. What is clear from the data so far is the complex nature of communities involved in methane metabolism. These communities are represented, in addition to *bona fide* methantrophs (the *Methylococcaceae* species), by methylotrophic species within the family *Methylophilaceae* and non-methylotrophic species mainly belonging to the order *Burkholderiales* and to the phylum *Bacteroidetes*. Preliminary analysis of the metatranscriptomes indicates that the most highly transcribed genes in the microcosms are the ones encoding methane monooxygenase, the first enzyme in methane oxidation. Genes encoding methanol dehydrogenase, catalyzing oxidation of the immediate product of methane oxidation, are also among the most highly expressed genes in both *Methylococcaceae* and *Methylophilaceae*. *Methylobacter* and *Methylostenera* species in addition express respiratory denitrification functions (incomplete pathway in the former and complete pathway in the latter). The *Burkholderiales* also highly expresses the denitrification functions, along with acetate metabolism functions. The *Bacteroidetes* appear to represent the next tier in the food web, utilizing extracellular polymeric substances produced by *Methylococcaceae* and *Methylophilaceae*. The relationships between the core set of organisms active in methane oxidation are brought to the

next level of complexity by the presence and activity of predatory species, among which *Bdellovibrionales* and *Myxococcales* dominate.

The bottom-up approach provides further insights into the metabolic interconnections between major functional guilds. Comparative transcriptomics of model *Methylobacter* and *Methylothera* species cultivated either axenically or as parts of two-species stable communities revealed differential expression of specific functions, suggesting their involvement in molecular mechanisms of interspecies metabolic/regulatory interdependence. We observed especially dramatic response of alternative methanol dehydrogenases to the cultivation conditions as follows. While in *Methylobacter* cultivated axenically, the XoxF type (lanthanide-dependent) methanol dehydrogenase was preferentially expressed, in cocultures, the MxaFI type (calcium-dependent) enzyme was preferentially expressed. The *Methylothera* species that tend to encode multiple XoxF enzymes differentially expressed different variants in axenic versus coculture conditions. The physiological meaning of differential choices of methanol-oxidizing enzymes in both partners is currently being addressed via the analysis of knock-out mutants in respective genes.

While our two-species synthetic community experiments concentrated so far on microcosms involving the most prominent *Methylococcaceae* partner, *Methylobacter*, as determined through the top-down approach, manipulation of synthetic communities of increased complexity used multiple (up to 50) species of methanotrophs, non-methanotrophic methylotrophs as well as non-methylotrophic heterotrophs. This experimental setup involves significantly more complexity compared to the two-species communities. However, these communities are completely tractable as they are made up of organisms with known genomic sequences, with predicted physiological traits validated through phenotypes observed in the lab, and these are mixed at predetermined relative abundances. By placing these communities under specific cultivation regimens ('low' versus 'high' oxygen, 'low' versus 'high' methane tensions etc.), we observed specific community dynamics, selecting for a smaller subset of originally mixed species, akin to dynamics observed in the top-down experiments. However, the species dominating these dynamics differed somewhat from the ones in the top-down experiments involving natural sediment communities. Most remarkably, *Methylomonas* species, while rapidly outcompeted in the top-down experiments with natural sediment samples by the *Methylobacter* species, persisted under both 'low' and 'high' oxygen pressures in these experiments, while *Methylosarcina* species persisted under 'low' methane. The nature of differential competitiveness of different *Methylococcaceae* species is being further addressed by employing two to three species communities of methanotrophs representing three major genera, *Methylobacter*, *Methylosarcina* and *Methylomonas*, and through increasing complexity of these communities by adding select non-methanotrophs species in different combinations.

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