

Novel Insights into Rare Earth-Mediated Metabolisms, Aerobic Methanotrophy under Hypoxia, and the Role of Nitrogen Species in Methane Oxidation

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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. Through manipulation of synthetic communities and systems biology approaches, we are striving to understand the molecular mechanisms that form a basis for interspecies interactions in microbial oxidation of methane. In this phase of the project our goals were: 1) Expand the biochemical knowledge on rare earth alcohol dehydrogenases in keystone methylotroph species and beyond, through enzyme purification and analysis; 2) Evaluate response of methane-oxidizing communities to hypoxia, pinpoint main mechanisms for adaptation, and test their significance via mutation; and 3) Directly address the interplay between denitrification and methane oxidation via mutant manipulation.

Through protein expression and analysis, we more than doubled the extant collection of the biochemically characterized rare earth-dependent enzymes, demonstrating a range of catalytic properties and substrate and cofactor specificities. Many of these enzymes reveal propensity for oxidation of methanol. This observation, in combination with genome-based reconstruction of methylotrophy pathways in select species suggests a much wider occurrence of this metabolic capability among bacterial species, and thus further suggests the importance of methylated compounds as parts of the global carbon cycle. We also compiled an inventory of genes potentially encoding rare earth-dependent enzymes closely related to the characterized enzymes, demonstrating their wide distribution among some of the most numerically abundant and environmentally important taxa, suggesting that reliance on rare earth-mediated biochemistries is much more widespread in the microbial world than previously assumed. Overall, our new data both firmly establish rare earth elements as the essential life metals and suggest their universal role in metabolisms that drive major biogeochemical processes, including but not limited to the conversion of methanol.

Through comparative (meta)transcriptomics, we identified potential mechanisms for hypoxia tolerance by *Methylobacter*, a globally occurring cosmopolitan species playing key role in methane consumption in oxic, hypoxic and even anoxic environments. We zoomed into one gene cluster that encodes a hybrid cluster protein and cognate oxidoreductase, along with regulatory proteins, unique to *Methylobacter* species. Mutations in these genes lead to decreased growth with nitrate but not ammonium, suggesting a role for these genes in nitrosative stress.

The role of denitrification was directly tested via mutation, again revealing decreased growth of the mutant in respiratory nitrate reductase, despite low expression of the denitrification genes under all conditions tested.

Overall, our new data provide additional insights into the functionality of methane-oxidizing communities and reveal physiological mechanisms that may be responsible for the dominant role of *Methylobacter* in methane cycle in natural environments with high fluxes of methane, including hypoxic and even anoxic environments. The source of dioxygen for methane activation under the latter condition remains obscure and requires further experimentation.

Publications

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