Competition Between Methanotrophs for Copper

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Project Goals: The overall goal of this project is to determine how significant microbial competition for copper is *in situ*, particularly how such competition affects net methane and nitrous oxide emissions. By better understanding how microbes compete for trace nutrients (i.e., copper) at a molecular level, we can scale such competition to ecosystem functioning, i.e., how microbial competition can be modeled to predict emerging microbial community composition and activity.

Abstract
Aerobic methanotrophs - microorganisms that oxidize methane by coupling it to dioxygen reduction - play a critical role in the biogeochemical cycling of carbon. More specifically, these intriguing microbes consume substantial amounts of methane generated via methanogenesis, and thus are important "filters" that control environmental emissions of methane. Expression and activity of alternative forms of methane monooxygenase (MMO, responsible for the conversion of methane to methanol), however, is controlled by copper, or the canonical "copper-switch"

Methanotrophs have been found to have multiple mechanisms of copper uptake. The first well-characterized copper binding compound or chalkophore – methanobactin – was found to be expressed by some methanotrophs of the *Methylocystaceae* family within the Alphaproteobacteria, e.g., *Methylosinus trichosporium* OB3b. Methanobactin (MB) is a modified polypeptide containing two heterocyclic rings with associated thioamide groups that collectively are responsible for copper binding with extremely high affinity. Not all methanotrophs, however, can produce MB. Rather, methanotrophs of the *Methylococcaceae* family of the Gammaproteobacteria rely on an outer membrane protein (MopE or CorA) for copper sequestration as well as some *Methylococcaceae* secreting a copper-binding compound akin to MB, but with much weaker affinity for copper.

Given the importance of copper in methanotrophy, this raises several intriguing questions. First, do methanotrophs that express MB have a competitive advantage for copper sequestration? Competition between methanotrophs for copper is likely, with such competition affecting overall methanotrophic community composition, and by extension methanotrophic activity. Second, given that MB is secreted into the environment and then taken up after binding copper, can copper-MB complexes be "stolen" by other microbes? Such phenomena would require non-MB expressing methanotrophs to have the uptake system identified for MB, i.e., the TonB-dependent transporter required for MB uptake (MbnT).

Herein we show that, similar to that found for siderophore theft between certain microbes, "cheating" exists between methanotrophs for MB. Specifically, *Methylomicrobium album* BG8 and *Methylocystis* sp. Rockwell, lacking genes for MB biosynthesis, carry genes for the TonB-dependent transporter required for MB uptake. Growth and gene expression studies show that these methanotrophs are not starved for copper in the presence of MB, but are in the presence of triethylenetetramine, a strong abiotic copper chelating agent. In addition, *Methylocystis* sp. Rockwell more readily uptakes a specific type of MB, indicating presence of a specific MbnT. Such data indicate that copper-MB complexes were taken up by *M. album* BG8 and *Methylocystis* sp. Rockwell. These results also indicate MB may be considered to be a "public good" and provides a new mechanism to manipulate microbial communities for control of greenhouse gas emissions *in situ*.

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