

Methanotrophs Produce Diverse Chalkophores to Compete 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

Methanotrophs, microorganisms that use methane as their sole source of carbon and electron, play a critical role in the biogeochemical cycling of carbon. These intriguing microbes consume substantial amounts of methane and thus serve as a significant sink for methane in the environment. Expression and activity of alternative forms of methane monooxygenase (MMO), which is responsible for the initial conversion of methane to methanol, is controlled by copper availability or the canonical “copper-switch”.

Methanotrophs have multiple mechanisms of copper uptake. Some methanotrophs of the *Methylocystaceae* family within the Alphaproteobacteria express a copper-binding compound or chalkophore – methanobactin (MB). MB is a small ribosomally synthesized, post-translationally modified polypeptide, that is characterized by two heterocyclic rings and associated enethiol groups that bind copper with extremely high affinity. Methanotrophs belonging to the Gammaproteobacteria, such as those of the *Methylococcaceae* family, rely on an outer membrane protein (MopE or CorA) for copper uptake, as well as some *Methylococcaceae* secreting a chalkophore similar to MB, but with much weaker affinity for copper.

Given the importance of copper in methanotrophy, it is highly likely that methanotrophs actively compete for copper, which raises an intriguing question. Since MB is secreted into the environment and copper-MB complexes are then taken up, can these complexes be “stolen” by other microbes, similar to siderophore theft observed between certain microbes?

Herein we show that both “cheating” and “competing” exist between methanotrophs for copper. Specifically, *Methylobacterium album* BG8, lacking genes for MB biosynthesis, carries a gene for TonB-dependent transporter required for MB uptake (*mbnT*). However, deletion of the putative *mbnT* does not affect the copper acquisition ability of *M. album* BG8, suggesting this gene is either not involved in MB uptake, or this microbe has an alternative pathway for copper

uptake. In addition, *M. album* BG8 is not starved for copper in the presence of MB, but is in the presence of triethylenetetramine (TRIEEN), an abiotic copper chelator. Specifically, *M. album* BG8 produces a MB-like substance when grown in the presence of both copper and TRIEN. Preliminary analyses of this potentially novel chalkophore indicated a size of approximately 1125 Da and characteristic UV-Vis spectra that are responsive to varying concentrations of copper. These results indicate that MB may be considered to be a “public good,” and that there may be yet to be characterized chalkophores that can affect environmental copper availability as well as microbial community composition and activity.

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