Metal Uptake by Methanotrophs: Genetic Basis for the Biosynthesis of A Novel Chalkophore and Molecular Spectroscopic Analyses of Mercury Detoxification

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http://sitemaker.umich.edu/methanobactin

Project Goals: Methanotrophs are ubiquitous in the environment, and despite their critical function in many different ecosystems, the biogeochemical factors that affect their activity and community structure are poorly understood. It is known that copper plays a key role in methanotrophic physiology, but the mechanism used by these microbes for copper acquisition was only recently discovered. This compound, methanobactin (mb), is the first example of a "copper-siderophore" or chalkopore. Mb binds many different metals, including mercury. Further, recent data show that different methanotrophs make different forms of mb that have varying metal affinities. The general objectives of this proposal are thus to consider how mb made by different methanotrophs alters the bioavailability of metals of concern to the DOE and how this affects: (1) the physiology, metabolism and gene expression in methanotrophs; (2) the broader microbial community structure and activity in laboratory soil columns, and; (3) the bioavailability of different metals in subsurface environments.

One of the persistent and substantial problems in remediation of hazardous waste sites is the mobilization and uncontrollable transport of radionuclides and heavy metals from these sites to surrounding areas. Some microbially-mediated processes can at least temporarily immobilize and reduce the toxicity of these materials through dissimilatory reduction that leads to precipitation and sorption under anaerobic conditions. As such, microbial-mediated processes can limit the dispersal of these materials and thus also limit the exposure of surrounding areas. Microorganisms, however, have effective and ubiquitous mechanisms to solubilize different metals and that non-specific binding of metals by these biogenic metal chelators may increase their solubility, mobility, and bioavailability. Here we are examining how the expression of metal chelating agents analogous to siderophores in methane-oxidizing bacteria i.e., methanotrophs, alters the bioavailability of various metals (copper and mercury) and how this: affects the (1) physiology, metabolism and gene expression in methanotrophs; (2) broader microbial community structure and meta-transcriptome, and; (3) bioavailability and risk associated with various metals. Such studies will enable us to determine how methanotrophic activity may affect the structure of subsurface microbial communities as well as the sustainability of subsurface waters, including at DOE sites.

Recent work in our laboratories has identified the genetic basis of mb and that many, but not all methanotrophs can synthesize mb. Interestingly, mb contains two heterocyclic rings, either imidazole, oxazolone or pyrazinedione rings with an associated enethiol group, which together are responsible for metal binding. Given the structure of the rings, it is quite possible that mb can also bind toxic metals such as mercury and that mb made by one methanotroph may affect the bioavailability of metals to other methanotrophs. Our findings show that mb from *Methylosinus trichosporium* OB3b does indeed bind mercury in addition to copper, and in doing so, reduced toxicity associated with Hg(II) to both α- and γ-Proteobacteria methanotrophs. Interestingly, mercury binding by mb was evident both in the presence and absence of copper, despite the fact that mb had a much higher affinity for copper due to the rapid and irreversible binding of mercury by mb. Metal analyses indicated that Hg(II), after bound by mb, may have been reduced to Hg(0) but was not volatilized. Rather, mercury remained associated with mb, and also was found associated with methanotrophic biomass. It thus appears, although the mercury-mb complex was cell-associated, mercury was not removed from mb.

It was also found that the amount of biomass-associated mercury in the presence of methanobactin from *M. trichosporium* OB3b was greatest for *M. trichosporium* OB3b and least for the tested γ-Proteobacteria methanotroph *(Methylomicrobium album* BG8), suggesting that methanotrophs may have selective mb uptake systems that may be based on TonB-dependent transporters, but that such uptake systems exhibit a degree of infidelity. Further, it was found that the addition of mb from *M. trichosporium* OB3b stimulated the growth of other methanotrophs in the absence of mercury but in the presence of copper. As methanotrophs expressing the particulate methane monooxygenase (the predominant form of methane monooxygenase expressed) require copper for high activity, it may be that methanobactin from *M. trichosporium* OB3b increased the bioavailability of copper, thereby increasing activity of pMMO in other methanotrophs. If so, this suggests that mb made by one methanotroph may actually be taken up by others (as also suggested by the mercury uptake data). Collectively, these studies raise several interesting questions, including do all methanotrophs in a mixed community produce mb, or do some species act as "cheaters" and rely on mb made by other microbes to meet copper requirements for metabolism or for detoxification of metals such as mercury? How do methanotrophs that make mb ensure that they are able to effectively compete with such cheaters for copper? It appears that the mb uptake systems have some infidelity, and that *in situ*, such infidelity may be optimized as a general competition strategy.

Ongoing work is characterizing the transcriptome of both α- and γ-Proteobacteria methanotrophs under a range of copper concentrations to determine how copper affects overall gene expression in methanotrophs. We have also sequenced the genome and transcriptome of the novel facultative methanotroph, *Methylocystis* strain SB2, grown with different carbon sources. We have also recently collected soil samples from the Integrated Demonstration Site of the DOE Savannah River Site where methanotrophs are known to exist. We are in the process of characterizing the initial microbial community composition and will also construct a series of soil columns to characterize how mb affects: (1) copper and mercury mobility and bioavailability in the presence of soils from this site, and; (2) dissolution of soil-associated minerals. The resultant effects on the broader microbial community structure and function will be determined via metagenomics and metatranscriptomics.

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This research was supported by the Office of Science (BER), U.S. Department of Energy.