Final Technical Report DOE Grant Number: DE-SC0020174

Project Title: Microbial Competition for Copper: Impacts on Carbon and Nitrogen Cycling

Report Number: DOE-UMICHIGAN-0020174

Name of Recipient: University of Michigan

Principal Investigators: Jeremy D. Semrau and Alan A. DiSpirito

Date of Report: October 31, 2023

Period Covered by Report: 9/15/2019 – 9/14/2023

I. Executive Summary

Activities from this DOE sponsored project focused on two general questions: (1) how does competition between microbes for copper affect emissions of greenhouse gasses, especially methane and nitrous oxide? and (2) how does a novel copper-binding compound (methanobactin) made by some methane-oxidizing bacteria bind and reduce metals, as well as what are the regulatory elements involved in controlling methanobactin synthesis?

From these efforts, we have conclusively shown that microbial competition for copper does indeed exert significant control on microbial community composition, as well as affects how microbes produce and consume nitrous oxide. We have also developed much better insights into the basis for methanobactin activity, as well as how methanotrophs control its production.

II. Project Goals

Methanotrophs, or methane-oxidizing bacteria, 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, is the first example of a "copper-siderophore", or chalkophore. Like siderophores, recent data show that different methanotrophs make different forms of methanobactin that have varying metal affinities. The general objectives of this project thus included examining: (1) how methanobactin made by different methanotrophs alters the bioavailability of copper to not only different methanotrophs but also the broader microbial community and, as a result emissions of both methane and nitrous oxide, (2) greater biochemical characterization of methanobactin, with the surprising finding that methanobactin can oxidize water to form dioxygen (the liberated electrons are used to reduce metals after they are bound by methanobactin) and (3) the regulatory elements involved in controlling methanobactin production.

III. Introduction

Every organism must extract from its environment multiple substances critical for their growth if not survival, and microorganisms are no different. Microbes, however, must rely exclusively on molecular mechanisms to scavenge needed elements, especially various metals critical for the activity of various enzymes. Perhaps not surprisingly then, many microbes (especially bacteria) have been shown to have have very effective mechanisms to collect different metals, e.g., copper. Here we examined how the

expression of methanobactin, a biogenic copper-binding agent analogous to a siderophore, affects the ability of the broad microbial community to collect copper, and especially the ability of microbes to regulate methane and nitrous oxide emissions.

Methanobactins are copper-binding molecules produced by methane-oxidizing bacteria, or methanotrophs. Methanobactin is used by methanotrophs as the extracellular component of a copper acquisition system to acquire copper in a stable and non-toxic form. Methanobactins are characterized as being small (< 1300 Da) polypeptides with two heterocyclic rings each with an adjacent thioamide group. The N-terminal ring is an oxazolone, while the other is either another oxazolone or an imidazolone or pyrazinedione group. The best-characterized methanobactins to date are those from *Methylosinus trichosporium* OB3b and *Methylocystis* strain SB2 (Figure 1).

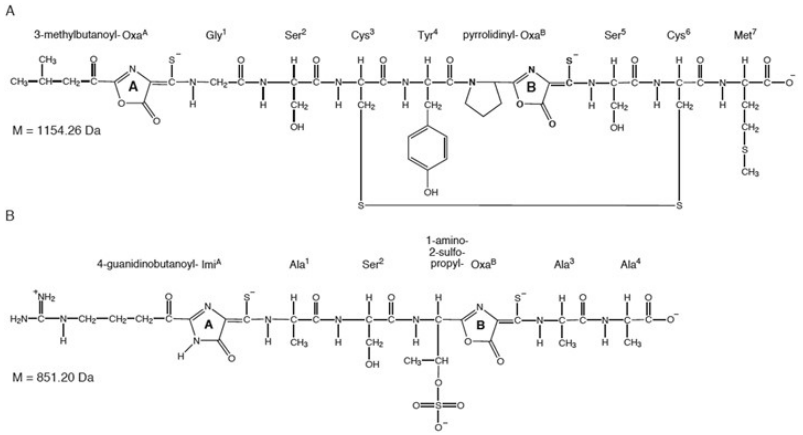

Figure 1. Primary structures of methanobactin from (A) *M. trichosporium* OB3b and (B) *Methylocystis* strain SB2.

Methanobactins have an extremely high copper-binding constant of ~$10^{18\text{-}58}$ M^{-1} [values vary depending on the method used to calculate affinity.

IV. Significant Accomplishments

Accomplishments in this project can be divided into the following categories

- Examination of copper competition between methanotrophs;
- Identification of a novel copper-binding compound expressed by at least one methanotroph
- Characterization of the sensitivity of denitrifiers to different forms of MB
- Characterization of the impact of MB and/or MB-expressing methanotrophs on greenhouse gas emissions from soils
- Characterization of the reductant source for metal reduction mediated by MBs
- Serendipitous discovery of iron-binding and solubilization by one form of MB that has potential medical applications, and
- Characterization of the regulatory elements involved in controlling MB expression

These are described in more detail below.

IV.A. Copper competition between methanotrophs. Aerobic methanotrophs utilize methane as their sole source of carbon and electron and play a critical role in mitigating methane emissions. Expression and activity of alternative forms of methane monooxygenase, which is responsible for the initial conversion of methane to methanol, is controlled by copper availability or the canonical "copper-switch." Methanotrophs have been found to have multiple mechanisms of copper uptake, such as the well-characterized copper binding compound or chalkophore – methanobactin (MB). MB is a modified polypeptide with extremely high affinity for copper. However, not all methanotrophs can produce MB, as only some methanotrophs of the *Methylocystaceae* family within the Alphaproteobacteria are known to produce MB. Given the importance of copper in methanotrophy, it was hypothesized that (1) competition between methanotrophs for copper is likely, and (2) methanotrophs lacking MB

biosynthesis genes may participate in piracy of copper-MB complexes as "cheaters" by expressing a TonB-dependent transporter (TBDT) required for MB uptake (MbnT). In this study, we found that *Methylomicrobium album* BG8, *Methylocystis* sp. strain Rockwell, and *Methylococcus capsulatus* Bath, all lacking genes for methanobactin biosynthesis, are not limited for copper by multiple forms of methanobactin. Interestingly, *Mm. album* BG8 and *Methylocystis* sp. strain Rockwell were found to have genes similar to *mbnT* that encodes for a TonB-dependent transporter required for methanobactin uptake. Data indicate that these methanotrophs "steal" methanobactin using these TonB-dependent transporters. *Mc. capsulatus* Bath, however, lacks anything similar to *mbnT* and data indicate it was unable to take up by any form of methanobactin tested. Rather, *Mc. capsulatus* Bath appears to rely on its own copper uptake system - MopE/MopE* - for copper collection, and this effectively competes with MB for copper. Finally, not only does *Mm. album* BG8 "steal" methanobactin, it synthesizes a novel chalkophore unlike any other identified copper-binding compound, suggesting that some methanotrophs utilize both competition and cheating strategies for copper collection.

In addition to examining MB uptake by non-NB producers, we examined the specificity of TBTDs in taking up different forms of MB. That is, we found that the model methanotroph, *Methylosinus trichosporium* OB3b, not only produces and collects its own form of MB, this methanotroph also will actively "steal" non-native MB through the use of a specific TBTD. Interestingly, in *Methylosinus trichosporium* OB3b, expression of this TBDT is induced in the presence of the non-native MB, but expression of the TBDT required for uptake of native MB is not induced in the presence of native MB, suggesting that methanotrophs actively seek to "steal" MB from other methanotrophs. Interestingly, the canonical "copper-switch" in *Ms. trichosporium* OB3b that controls expression of alternative MMOs is apparent if the TBDT required for either form of MB is disrupted, but is "broken" if both TBTDs are disrupted. These data indicate that MB uptake plays an important role in the copper switch in *Ms. trichosporium* OB3b, and we have developed a new model for the "copper-switch" in this methanotroph that involves MB.

IV.B. Identification of a novel copper-binding compound expressed by Methylomicrobium album BG8.

Preliminary data indicated that *Methylomicrobium album* BG8, unable to produce MB, synthesized some novel yet unknown copper-binding compound. To investigate this further, *Mm. album* BG8 was grown in the presence of triethylenetetramine (TRIEN), a strong abiotic chelator of copper, to determine if copper limitation could induce overproduction of this chalkophore. When *Mm. album* BG8 was grown in the presence of 1 μM copper + 5 μM TRIEN, growth was visibly reduced and the spent medium became yellow. Such coloration was not observed when *Mm. album* BG8 was grown in the presence of copper, indicating that *Mm. album* BG8 secretes some yellowish substance when copper availability is reduced through the addition of TRIEN. To isolate this putative novel chalkophore produced by *Mm. album* BG8, supernatant of this methanotroph grown in the presence of copper and TRIEN was collected and filtered through a 0.2-μm PES filter unit. A reversed-phase C_{18} Sep-Pak cartridge was sequentially conditioned with 3 mL methanol, 3 mL 60% acetonitrile, 3 mL methanol, and 6 mL H_2O, and then loaded with the filtered spent medium. The chalkophore bound to the column was washed with H_2O, then eluted with 60% acetonitrile until a yellow band was collected. The eluant was frozen at -80 °C and lyophilized for chalkophore concentration and removal of acetonitrile. The chalkophore was isolated from the spent media and purified via a Targa C18 column using a methanol : H_2O gradient. UV-visible spectroscopy, matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) and electrospray ionization (ESI)MS/MS assays were performed to characterize this compound. This putative chalkophore was found to have a molecular mass of 649.95 or 653.29 Da as

determined by MALDI-MS or ESI-MS, respectively. Following the addition of $CuCl_2$ the molecular mass shifted to 711.35 and 713.35 Da as determined by MALDI-MS or ESI-MS, indicating that this substance indeed binds copper (i.e., is a chalkophore), but likely loses 2 or $3H^+$ following copper binding. The UV-VIS spectrum of the isolated chalkophore did not have the characteristic peaks present in MBs (i.e., at ~340 and 394 nm), but did exhibit distinct absorption maxima at 396 nm and 402 nm with a molar extinction coefficient of 1.6 $mM^{-1}cm^{-1}$ at 402 nm . A discrete isosbestic point at 340 nm at mole ratios of copper:chalkophore between 0 and 0.19 was evident. Absorption maxima shifted to 404 nm at copper:chalkophore ratios between 0.2 and 0.45 with a discrete isosbestic point at 398 nm and to 390 nm at Cu to chalkophore ratios above 0.6. Growth and copper uptake studies of *Mm. album* BG8 shows that this chalkophore enables *Mm. album* BG8 collect copper in the presence of MB.

IV.C. Characterization of the sensitivity of denitrifiers to different forms of MB. There are multiple forms of MB, with two major groups found with significant structural and compositional differences between these groups. More specifically, as shown in Figure 1, MB can be divided into two general groups – Group I and II. Group I MBs have two oxazolone rings and form a dicyclic pyramidal-like shape after binding copper, e.g., MB from *M. trichosporium* OB3b (MB-OB3b). Group II MBs have one oxazolone ring with the second ring either an imidazolone or pyrazinedione ring, and adopt a hairpin-like shape after binding copper, e.g., MB from *Methylocystis* sp. SB2 (MB-SB2). Previous work on MB effect on dentrifier activity utilized MB-OB3b, i.e., a Group I MB. In these studies, it was found that irreversible inhibition of NosZ in both *Pseudomonas stutzeri* DCP-Ps1 and *Dechloromonas aromatica* RCB was only observed in the presence of 1 µM MB-OB3b. We repeated these studies with all conditions identical with the exception that MB-SB2 (a Group II MB) was substituted for MB-OB3b. Here complete inhibition of NosZ was found when 500 nM MB-SB2 was added, indicating that different forms of MB have significantly different impacts on denitrifier activity.

IV.D. Characterization of MB-expressing methanotrophs in soils and their impact on greenhouse gas emissions. The possibility that copper sequestration strategies of methanotrophs may interfere with copper uptake of denitrifiers *in situ* and thereby enhance N_2O emissions (due to inhibited activity of the nitrous oxide reductase, NosZ, that requires copper) was also examined. Addition of purified methanobactin from *Methylosinus trichosporium* OB3b or *Methylocystis* species strain SB2 to denitrifying soil microbial consortia resulted in substantially increased N_2O production, with more pronounced responses observed for soils with lower copper content. As expected from pure culture studies, MB from *Methylocystis* sp. strain SB2 had a more pronounced effect and caused greater accumulation of nitrous oxide Further, the presence of either form of MB also inhibited nitrite reduction and generally increased the presence of genes encoding for the iron-containing nitrite reductase (*nirS*) over the copper-dependent nitrite reductase (*nirK*). These data indicate the methanotrophic-mediated production of MB can significantly impact multiple steps of denitrification, as well as have broad effects on microbial community composition of forest soils. The N_2O emission-enhancing effect of the soil's native *mbnA*-expressing *Methylocystaceae* methanotrophs on the native denitrifiers was also experimentally verified with a *Methylocystaceae* dominant chemostat culture prepared with a soil microbial consortium as the inoculum. Lastly, with the microcosms amended with varying cell numbers of methanobactin-producing *Methylosinus trichosporium* OB3b before CH_4 enrichment, microbiomes with different ratios of methanobactin producing *Methylocystaceae* to gammaproteobacterial methanotrophs incapable of methanobactin production were simulated. Significant enhancement of N_2O production from denitrification was evident in both *Methylocystaceae*-dominant and *Methylococcaceae*-dominant enrichments, albeit to a greater extent in the former, signifying the comparative potency of methanobactin-mediated copper sequestration while implying

the presence of alternative copper abstraction mechanisms for *Methylococcaceae*. These observations support that copper-mediated methanotrophic enhancement of N_2O production from denitrification is plausible where methanotrophs and denitrifiers cohabit.

IV.E. Characterization of the reductant source for metal reduction mediated by MBs. MBs can bind a range of metals, with some reduced after binding, e.g., Cu^{2+} is reduced to Cu^{+} and Au^{3+} to Au^{0}. Other metals, however, are bound but not reduced, e.g., Co^{2+} and K^{+}. The source of electrons for selective metal reduction has been speculated to be water but never empirically shown. Here, using $H_2{}^{18}O$, we found that when MB from *Methylocystis sp*. strain SB2 (MB-SB2) and *Methylosinus trichosporium* OB3b (MB-OB3) were incubated in the presence of either Au^{3+}, Cu^{2}, and Ag^{+}, $^{18,18}O_2$ and free protons were released. No $^{18,18}O_2$ production was observed either in presence of MB-SB2 or MB-OB3b alone, gold alone, copper alone, silver alone or when K^{+} or Mo^{2+} was incubated with MB-SB2. In contrast to MB-OB3b, MB-SB2 binds Fe^{3+} with an N_2S_2 coordination and will also reduce Fe^{3+} to Fe^{2+}. Iron reduction was also found to be coupled to oxidation of $2H_2O$ and generation of O_2. MB-SB2 will also couple Hg^{2+}, Ni^{2+} and Co^{2+} reduction to the oxidation of $2H_2O$ and generation of O_2, but MB-OB3b will not, ostensibly as MB-OB3b binds but does not reduce these metal ions. Such a finding is especially intriguing as it suggests that methanotrophs expressing MB may be able to maintain their activity in hypoxic/anoxic conditions through "self-generation" of dioxygen required for the initial oxidation of methane to methanol. Indeed, methanotrophs capable of expressing MB are commonly reported to prefer high methane/low oxygen environments, and are also found in anoxic areas. Thus, the ability to express MB may be an important factor in enabling methanotrophs to not only colonize the oxic-anoxic interface where methane concentrations are highest, but also tolerate significant temporal fluctuations of this interface due to precipitation. To determine if the O_2 generated during metal ion reduction by MB could be coupled to methane oxidation, $^{13}CH_4$ oxidation by *Methylosinus trichosporium* OB3b was monitored under anoxic conditions. The results demonstrate O_2 generation from metal ion reduction by MB-OB3b can support methane oxidation. These results have significant implications for our understanding of modern microbial ecology of methanotrophs how they can be manipulated to control methane emissions *in situ*, as well as the evolution of methanotrophy.

IV.F. Serendipitous discovery of iron-binding and solubilization by one form of MB. As part of our work characterizing the source of electrons for MB-mediated metal reduction we unexpectedly discovered that of the two general forms of MB (Group I and II), at least one Group II MB produced by *Methylocystis* sp. strain SB2 will bind and reduce Fe(III) to Fe(II), and in so doing, will dissolve insoluble forms of iron. Further, this form of methanobactin has been found to increase fecal excretion of iron from model rat organisms and as such may have therapeutic value in treatment of iron-related human diseases, e.g., hemochromatosis (note that the animal studies were done by a Hans Zischka, a collaborator in Germany). This disorder causes the body to absorb excess iron, particularly in the liver and heart. Left untreated or upon compliance issues, hemochromatosis can and is often fatal. Further, methanobactin may be useful in treating acute iron overload that occurs due to excess consumption of iron supplements. We have previously shown that methanobactin is a very promising treatment of Wilson's Disease, a disease where the body is unable to correctly assimilate copper (such technology has been licensed to ARBORMED). Thereat, methanobactin most efficiently depleted liver copper overload within days of treatment down to wild-type levels, allowing weeks of treatment pause. As such, we believe that there is significant potential to expand the utility of methanobactin to include the treatment of iron-overload disorders. Iron overload can occur among patients with hereditary hemochromatosis, thalassemia, sickle cell disease, aplastic anemia, myelodysplasia, and other diseases. Hereditary hemochromatosis is due to mutations in genes encoding proteins involved in limiting

systemic iron uptake. Around 10% of the population are heterozygous carriers and 0.3-0.5 % are homozygous. Currently, these diseases, which are caused by dysregulated iron uptake, are treated by regular bleeding (up to 500ml/per week) or iron chelators like deferiprone, deferasirox, and deferoxamine. These chelators have unwanted side effects and are less effective than bleeding. To our knowledge they are only partially able to dissolve iron deposits. We have recently filed a US Provisional Patent Application, No. 63/144,338 "Use of methanobactin for treatment of iron-related diseases" on this discovery.

IV.G. Characterization of the regulatory elements involved in controlling MB expression. As discussed previously, copper is well-known to control the activity of methanotrophy, and that many methanotrophs rely on methanobactin for copper collection. It has also been found that methanotrophs that express MB due so only when copper is limiting, i.e., copper bioavailability is low. What is not known is what regulatory elements are involved in ensuring methanobactin is only expressed when needed to scavenge copper. As part of our work on this project, we found that MmoD, a gene suspected to play a role in the "copper-switch" in *M. trichosporium* OB3b is critical for controlling methanobactin production in this methanotroph. That is, when *mmoD* was deleted, MB expression was no longer repressed by the availability of copper, and rather production of MB increased substantially over wildtype *M. trichosporium* OB3b. These results thus provide improved insight into the components of the "copper-switch" as well as provide new strategies to manipulate methanotrophic activity to not only reduce methane emissions, but also remove methane from the atmosphere. In addition, these findings suggest strategies to enhance methanobactin production, for as mentioned earlier, it has promise for the treatment of a variety of metal-related human diseases. We have recently filed a patent application for this latter purpose.

V. Personnel Hired

At the University of Michigan, two post-doctoral scholars were supported as part of this project: Dr. Peng Peng and Dr. Jin Chang. Further, one graduate student was engaged at the University of Michigan as part of this project, Christina Kang-Yun (graduated June 2021). At Iowa State University, one graduate student, Philip Dershwitz (graduated May 2022) and two undergraduate students, Joshua. C. Ledesma and Daly J. Pelger were engaged as part of the project.

VI. Products

VI.A. Peer-reviewed articles in archival journals

- Graduate students listed among the authors are double underlined.
- Undergraduate students are single underlined and noted by an asterisk * after their name.
- Corresponding author is noted by §.

1. "MmoD regulates soluble methane monooxygenase and methanobactin production in *Methylosinus trichosporium* OB3b" P. Peng, J. Yang, A. A. DiSpirito, and J. D. Semrau§. Accepted, *Applied and Environmental Microbiology*.
2. "Inhibition of Nitrous Oxide Reduction in Forest Soil Microcosms by Different Forms of Methanobactin". J. Chang, P. Peng, M. Farhan Ul Haque, A. Hira, A. A. DiSpirito, and J. D. Semrau§. Accepted, *Environmental Microbiology*.
3. "Crystal structure of MbnF: a NADPH-Dependent Flavin Monooxygenase from *Methylocystis* strain SB2". A. Stewart, P. Dershwitz, C. Stewart, M. Sawaya, T. Yeates, J. Semrau, H. Zischka, A. DiSpirito and T. Bobik§. *Structural Biology Communications*. 79:111-118. 2023.

4. "Multiple mechanisms for copper uptake by *Methylosinus trichosporium* in the presence of heterologous methanobactin" P. Peng, W. Gu, A. A. DiSpirito, and J. D. Semrau[§]. *mBio*. 13:e02239-22. 2022.
5. "Updated Genome Sequence of the Facultative Methanotroph *Methylocystis* sp. Strain SB2". C. S. Kang-Yun, J. Chang, and J. D. Semrau[§]. *Microbiology Resource Announcements*. e00188-22. 2022.
6. "Variable Inhibition of Nitrous Oxide Reduction in Denitrifying Bacteria by Different Forms of Methanobactin". J. Chang, P. Peng, A. A. DiSpirito, J. D. Semrau[§]. *Applied & Environmental Microbiology*. e02346-21. 2022.
7. "MbnC is not required for the formation of the N-terminal oxazolone in methanobactin from *Methylosinus trichosporium* OB3b" P. Dershwitz, W. Gu, J. Roche, C. S. Kang-Yun, J. D. Semrau, T. A. Bobik, B. Fulton, H. Zischka, and A. A. DiSpirito[§]. *Applied & Environmental Microbiology*. e01841-21. 2022.
8. "Two TonB-dependent transporters in *Methylosinus trichosporium* OB3b are responsible for uptake of different forms of methanobactin and are involved in the canonical 'copper switch'". P. Peng, C. S. Kang, J. Chang, W. Gu, A. A. DiSpirito, J. D. Semrau[§]. *Applied & Environmental Microbiology*. e01793.2022.**NOTE: This article was selected by the Editor in Chief of *Applied & Environmental Microbiology* to be a "Spotlight Article", i.e., an article of significant interest to the readers of *Applied & Environmental Microbiology.***
9. "Evidence for Methanobactin "Theft" And Novel Chalkophore Production in Methanotrophs: Impact on Methanotrophic-Mediated Methylmercury Degradation". C. S. Kang-Yun, X. Liang, P. Dershwitz, W. Gu, A. Schepers, A. Flatley, J. Lichtmannegger, H. Zischka, L. Zhang, X. Lu, B. Gu, J. C. Ledesma*, D. J. Pelger*, A. A. DiSpirito, and J. D. Semrau[§]. *The ISME Journal*. 16:211-220. 2022. **NOTE: Selected by the Editor in Chief of *The ISME Journal* to be especially highlighted through the commission of a peer-reviewed commentary by an objective third party.**
10. "Spectroscopic and Computational Investigations of Organometallic Complexation of Group 12 Transition Metals by Methanobactins from *Methylocystis sp.* SB2." P. Eckert, A. Johs, J. D. Semrau, A. A. DiSpirito, R. Sarangi, J. Richardson, B. Gu, and E. M. Pierce. *Journal of Inorganic Biochemistry*. 223:111496. 2021.
11. "Oxygen generation via water splitting by a novel biogenic metal binding compound." P. Dershwitz, N. L. Bandow, J. Yang, J. D. Semrau, M. T. McEllistrem, R. A. Heinze, M. Fonseca, A. M. DiSpirito, N. S. Athwal, M. S. Hargrove, T. A. Bobik, and A. A. DiSpirito[§]. *Applied & Environmental Microbiology.* e00286-21. 2021
12. "Enhancement of nitrous oxide emissions in soil microbial consortia via copper competition between proteobacterial methanotrophs and denitrifiers". J. Chang, D. Kim, J. D. Semrau, J. Lee, H. Heo, W. Gu, and S. Yoon[§]. *Applied & Environmental Microbiology.* e02301-20. 2021.
13. Methanobactin from methanotrophs: Genetics, Structure, Function and Potential Applications". J D. Semrau[§], A. A. DiSpirito, P. K. Obulisamy[1], and C. S. Kang-Yun. *FEMS Microbiology Letters*. 367: fnaa045. 2020

VI.B. PhD Theses

1. "Characterization of the molecular basis of regulation of gene expression by metals in methanotrophs". Christina Kang-Yun, The University of Michigan, June 2021.
2. "Methanobactin biosynthesis and redox activity" Philip Dershwitz, Iowa State University, May 2022.

VI.C. Oral Presentations

1. “Methane Removal Approaches Using Methanotrophic Bacteria”. Methane Emissions Alliance Technology, Stanford University. July 13, 2023.
2. “Synthesis of Current and Future Efforts to Mitigate Methane Emissions”. American Academy of Microbiology Workshop: The Role of Microbes in Mediating Methane Emissions – Act Today to Prepare for Tomorrow. June 1, 2023.
3. “3M: Methanotrophs, Metals, and Methanobactin”. UK EU C1 & BVOC Symposium. University of Warwick, May 10, 2023.
4. “Methanobactin: more than a metal binding peptide” UK EU C1 & BVOC Symposium. University of Warwick, May 10, 2023.
5. “Methanotrophy and Its Interactions with Metals: Implications and Applications. San Diego State University. November 3, 2022.
6. “Recent Musings in Methanotrophy”. Department of Biological Sciences. The University of Alberta. September 16, 2022.
7. “Copper Competition Between Microbes: Implications for Greenhouse Gas Emissions”. Biological and Environmental Research (BER) 2021 Genomic Sciences Program Annual PI Meeting. February 24, 2021. Washington DC.
8. “Environmental Implications of Metal Binding by the Novel Chalkophore Methanobactin”. Goldschmidt Virtual 2020, June 24, 2020.
9. “Microbial Competition for Copper: Impacts on Carbon and Nitrogen Cycling”. Biological and Environmental Research (BER) 2020 Genomic Sciences Program Annual PI Meeting. February 25, 2020. Washington DC.
10. “Genetics, Biochemistry and Applications of Methanotrophy”. Department of Civil, Environmental and Architectural Engineering. The University of Texas at Austin. Oct. 18, 2019.

VI.D. Tech Transfer Activities

1. US Provisional Patent Application No. 63/452,572. “Alternative Strategies for Methanobactin Production”. Filed March 16, 2023.
2. US. Provisional Patent Application No. 63/144,338 "Use of Methanobactin for Treatment of Iron-Related Diseases". Filed February 1, 2021.