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MINIREVIEW

# Metals and Methanotrophy

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**ABSTRACT** Aerobic methanotrophs have long been known to play a critical role in the global carbon cycle, being capable of converting methane to biomass and carbon dioxide. Interestingly, these microbes exhibit great sensitivity to copper and rare-earth elements, with the expression of key genes involved in the central pathway of methane oxidation controlled by the availability of these metals. That is, these microbes have a "copper switch" that controls the expression of alternative methane monooxygenases and a "rare-earth element switch" that controls the expression of alternative methanol dehydrogenases. Further, it has been recently shown that some methanotrophs can detoxify inorganic mercury and demethylate methylmercury; this finding is remarkable, as the canonical organomercurial lyase does not exist in these methanotrophs, indicating that a novel mechanism is involved in methylmercury demethylation. Here, we review recent findings on methanotrophic interactions with metals, with a particular focus on these metal switches and the mechanisms used by methanotrophs to bind and sequester metals.

**KEYWORDS** methanotrophy, methanobactin, copper, mercury, rare-earth elements

Aerobic methane-oxidizing bacteria, i.e., methanotrophs, are an intriguing group of microorganisms that utilize methane as their sole source of carbon and energy. There is great interest in these microbes, as they (i) are found wherever methane-air interfaces develop, (ii) are phylogenetically diverse, (iii) play important roles in the global carbon cycle, (iv) have been extensively used for the biodegradation of halogenated hydrocarbons, and (v) have great potential in promoting sustainability by valorizing methane to biofuels, bioplastics, and osmoprotectants, among other products (1–5).

Remarkably, aerobic methanotrophs exhibit multiple "metal switches," where gene expression is controlled by the availability of metals, particularly copper and rare-earth elements. Further, methanotrophs have been recently shown, via the involvement of the chalkophore methanobactin, to alter the speciation and availability of other metals, including mercury. In this minireview, we provide an overview of how metals affect aerobic methanotrophy, as well as the potential applications of methanotrophy.

## OVERVIEW OF METHANOTROPHY

Aerobic methanotrophs are commonly found at oxic-anoxic interfaces of terrestrial, marine, and freshwater environments where they feed on methane produced by methanogens in anoxic zones (6). Methanotrophs can also thrive in extreme environments, including those that are acidophilic, alkaliphilic, thermophilic, or psychrophilic (2, 7–14). Finally, methanotrophs have been isolated from the phyllosphere (15–18), indicating that they are widespread in nature.

Most aerobic methanotrophs group with either the *Alphaproteobacteria* or *Gammaproteobacteria*, now with more than 20 genera combined in the two classes (Fig. 1).

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This article is dedicated to the memory of Stephen H. Stanley and Howard Dalton, leading researchers in methanotrophy who deduced the copper switch in 1983.

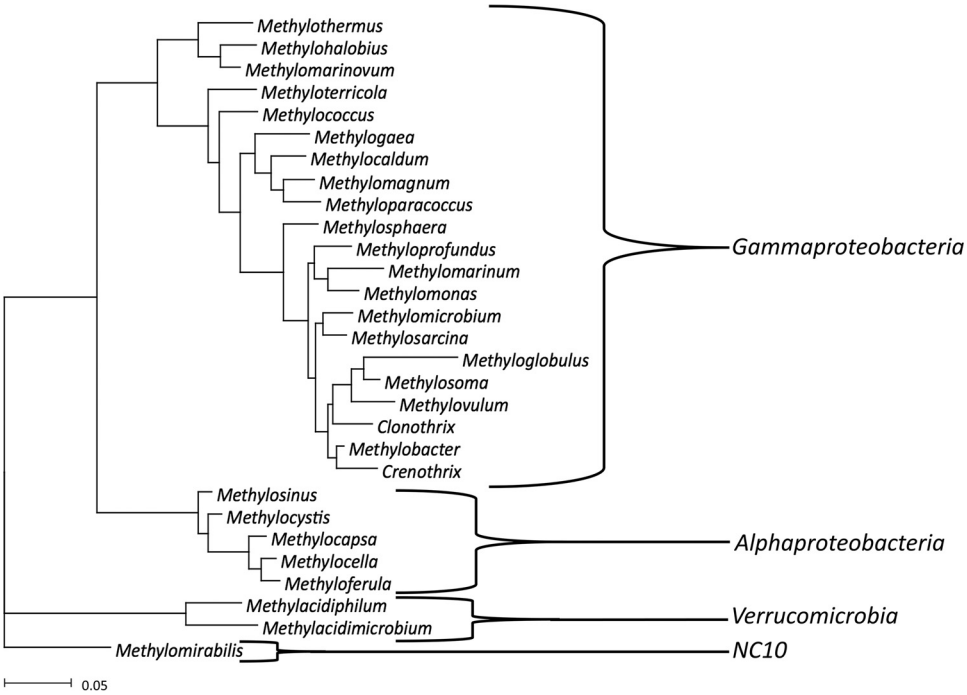


**FIG 1** Phylogenetic distribution of aerobic methanotrophic genera. 16S rRNA sequence alignment was generated using the SILVA aligner (164) and used to construct a maximum likelihood tree based on the Tamura-Nei model in MEGA7 (165). For the sake of brevity, methanotrophic species are not listed. For a thorough list/description of validated aerobic methanotrophic species, the reader is directed to reference 6. It should be noted that to date, no type strains have been isolated/purified from the *Clonothrix*, *Crenothrix*, or *Methylomirabilis* genus.

Recently, however, the isolation of thermoacidophilic methanotrophs that represented distinct lineages within the *Verrucomicrobia* phylum has been reported, i.e., the *Methylacidiphilum* and *Methylacidimicrobium* genera (2, 7, 8, 11, 14, 19). Another novel methanotroph is "*Candidatus* Methylomirabilis oxyfera" (belonging to the bacterial NC10 phylum), which has the unique ability to couple methane oxidation to nitrite reduction, and in so doing generate dioxygen required for the initial turnover of methane to methanol (20).

Despite the broad phylogenetic and environmental distribution of aerobic methanotrophs, the pathways employed by these microbes are remarkably similar, with four general steps, as shown in Fig. 2. Methane is first converted to methanol by methane monooxygenase (MMO), which is then oxidized to formaldehyde by methanol dehydrogenase (MeDH). Formaldehyde can then be converted to formate via either the tetrahydrofolate or tetrahydromethanopterin pathway and then can be oxidized to carbon dioxide by formate dehydrogenase. Most aerobic methanotrophs take up carbon at the level of formaldehyde via either the ribulose monophosphate or serine cycle (5). Some methanotrophs, however, do not utilize either pathway for carbon assimilation. Rather, some methanotrophs fix carbon dioxide via the Calvin-Benson-Bassham (CBB) cycle, i.e., *Methyloacidiphilum fumariolicum* SolV, *Methylacidimicrobium* spp., and "*Candidatus* Methylomirabilis oxyfera" (19, 21, 22). Interestingly, genomes of some *Proteobacteria* methanotrophs have been found to encode the complete CBB cycle, e.g., *Methylococcus capsulatus* Bath, *Methylocapsa palsarum*, and *Methyloferula stellata* (23–25). These microbes, however, apparently cannot solely utilize the CBB pathway for carbon assimilation.

## ELUCIDATION OF THE COPPER SWITCH IN METHANOTROPHS AND MECHANISM OF COPPER UPTAKE

Two distinct forms of MMO have been characterized: a cytoplasmic or soluble methane monooxygenase (sMMO) and a membrane-bound or particulate methane

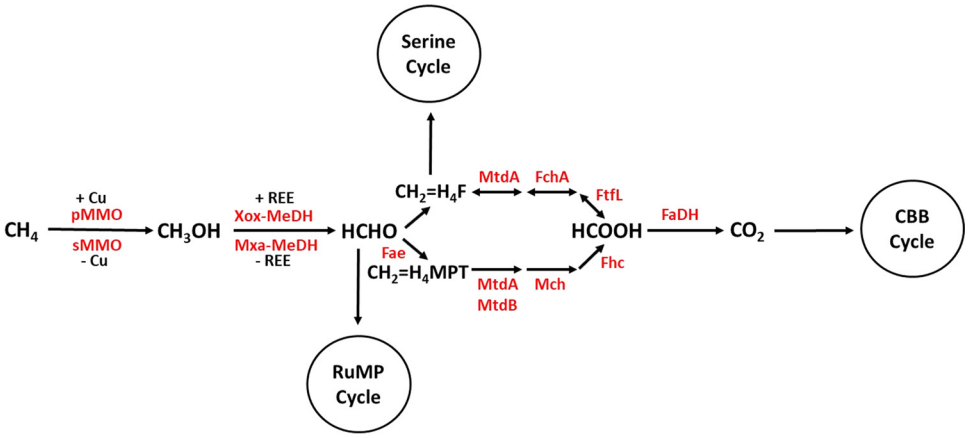


**FIG 2** General pathway of methane oxidation by aerobic methanotrophs. Enzymes are noted in red. pMMO, particulate methane monooxygenase; sMMO, soluble methane monooxygenase; Xox-MeDH, Xox-methanol dehydrogenase; Mxa-MeDH, Mxa-methanol dehydrogenase; Fae, formaldehyde-activating enzyme; $H_4$MPT, tetrahydromethanopterin; MtdB, NADP-dependent methylene-$H_4$MPT dehydrogenase; Mch, methenyl-$H_4$MPT cyclohydrolase; Fhc, formyltransferase-hydrolase complex; $H_4$F, tetrahydrofolate; MtdA, NADP-dependent methylene-$H_4$F–methylene-$H_4$F dehydrogenase; FchA, methenyl $H_4$F-cyclohydrolase; FtfL, formate tetrahydrofolate ligase; FaDH, formate dehydrogenase; CBB, Calvin-Benson-Bassham; RuMP, ribulose monophosphate.

monooxygenase (pMMO). Some methanotrophs can express both forms of MMO, while others can only express pMMO, and some only have sMMO (Table 1). For those methanotrophs that can express both sMMO and pMMO, the expression of these forms is controlled by the availability of copper, i.e., the canonical "copper switch" (26). Under copper-deficient conditions, these methanotrophs will express sMMO, but as copper-to-biomass ratios increase, sMMO expression decreases substantially and pMMO expression increases. sMMO and pMMO have very different substrate ranges and kinetics; thus, it is important to understand the basis of the copper switch to exert greater control over methanotrophic activity. For example, pMMO has higher affinity for $CH_4$,

**TABLE 1** Distribution of methane monooxygenases, methanol dehydrogenases, and methanobactin in select methanotrophs as determined via genomic interrogation for *pmoA*, *mmoX*, *mxaFI*, *xoxF*, and *mbnBC*

| Phylum or class | Family | Strain (GenBank accession no.) | pMMO | sMMO | Mxa-MeDH | Xox-MeDH | MB |
|---|---|---|---|---|---|---|---|
| *Gammaproteobacteria* | *Methylococcaceae* | *Methylobacter tundripaludum* SV96 (NZ_AEGW00000000) | Yes | No | Yes | Yes | No |
| | | *Methylocaldum szegediense* O-12 (NZ_ATXX00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methylococcus capsulatus* Bath (NC_002977.6) | Yes | Yes | Yes | Yes | No |
| | | *Methylogaea oryzae* JCM 16910 (NZ_BBDL00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methyloglobulus morosus* KoM1 (NZ_AYLO00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methylomarinum vadi* strain IT-4 (JPON01000001.1) | Yes | No | Yes | Yes | No |
| | | *Methylomagnum ishizawai* strain 175 (FXAM00000000.1) | Yes | Yes | Yes | Yes | No |
| | | *Methylomicrobium album* BG8 (AFJF00000000.2) | Yes | No | Yes | Yes | No |
| | | *Methylomicrobium buryatense* 5G (AOTL00000000.1) | Yes | Yes | Yes | Yes | No |
| | | *Methylomonas* sp. LW13 (JNLB00000000.1) | Yes | Yes | Yes | Yes | No |
| | | *Methylomonas denitrificans* FJG1 (CP014476.1) | Yes | No | Yes | Yes | No |
| | | *Methylosarcina fibrata* AML-C10 (ARCU00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methyloprofundus sedimenti* strain WF1 (LPUF00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methyloterricola oryzae* strain 73a (JYNS00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methylovulum miyakonense* HT12 (AQZU00000000.1) | Yes | Yes | Yes | Yes | No |
| | *Methylothermaceae* | *Methylohalobius crimeensis* 10Ki (ATXB00000000.1) | Yes | No | Yes | Yes | No |
| *Alphaproteobacteria* | *Beijerinckiaceae* | *Methylocapsa acidiphila* B2 (ATYA00000000.1) | Yes | No | Yes | Yes | No |
| | | *Methylocella silvestris* BL2 (NC_011666.1) | No | Yes | Yes | Yes | No |
| | | *Methyloferula stellata* AR4 strain AR4T (ARWA00000000.1) | No | Yes | Yes | Yes | No |
| | *Methylocystaceae* | *Methylocystis* sp. strain SC2 (NC_018485.1) | Yes | No | Yes | Yes | Yes |
| | | *Methylocystis* sp. strain LW5 (JMKQ00000000.1) | Yes | Yes | Yes | Yes | Yes |
| | | *Methylosinus trichosporium* OB3b (ADVE00000000.2) | Yes | Yes | Yes | Yes | Yes |
| NC10 | Unclassified | "*Candidatus* Methylomirabilis oxyfera" (NSJN00000000.1) | Yes | No | Yes | Yes | No |
| *Verrucomicrobia* | *Methylacidiphilaceae* | *Methylacidiphilum fumariolicum* SolV (CAHT00000000.1) | Yes | No | No | Yes | No |

while sMMO has a higher maximum $CH_4$ turnover rate (27). sMMO also has a wider substrate range, being able to oxidize alkanes up to $C_8$, as well as ethers, cyclic alkanes, and aromatic hydrocarbons (28, 29). pMMO can oxidize alkanes up to $C_5$ but not aromatic compounds (29). Thus, when applying methanotrophs for the bioremediation of organic pollutants, one must carefully consider what compound(s) is (are) to be degraded. One must also consider long-term methanotrophic viability, as these pollutants typically cannot support methanotrophic growth. Methanotrophs expressing pMMO can be more advantageous than those expressing sMMO for pollutant degradation, i.e., as pMMO has greater specificity for $CH_4$, it is more effective at turning over the growth substrate (methane) in the presence of nongrowth substrates (i.e., pollutants). These nongrowth substrates are still oxidized, albeit at a lower rate (27, 30). As such, for some pollutants, it is more appropriate to utilize pMMO-expressing methanotrophs, as these cultures have greater activity over time due to the continued turnover of the growth substrate, methane. Therefore, long-term bioremediation strategies using methanotrophs should carefully consider what pollutant(s) is (are) to be targeted and provide growth conditions to ensure that the appropriate MMO is being expressed.

The copper switch can be explained in part from the metal composition of the two forms of MMO. sMMO is a soluble di-iron monooxygenase and is composed of a hydroxylase, reductase, and a regulatory subunit encoded by the *mmo* operon. Methane oxidation occurs at a bis-$\mu$-hydroxo-bridged di-iron center within the hydroxylase (31). pMMO, on the other hand, is an integral membrane metalloenzyme composed of three polypeptides arranged in an $\alpha_3\beta_3\gamma_3$ trimer (32–35) and is encoded by the *pmo* operon. There is some argument as to the active site of pMMO, with several competing models. One model proposes that methane oxidation occurs at a di-copper site in PmoB (the $\alpha$ subunit of pMMO [36]). Abiotic synthesis of a high-valent bis($\mu$-oxide) di-copper (III) complex structurally similar to the proposed di-copper site indicates that it may indeed be capable of oxidizing C-H bonds, with bond dissociation energies on the order of 75 kcal · $mol^{-1}$ (37), but this is less than the bond dissociation energy of the C-H bond in methane (104 kcal · $mol^{-1}$). Alternatively, density functional theory (DFT) and quantum mechanic/molecular mechanic (QM/MM) analyses suggest that mixed-valent bis($\mu$-oxo)Cu(II)Cu(III) and ($\mu$-oxo)($\mu$-hydroxo)Cu(II)Cu(III) complexes could abstract a hydrogen from methane (38). Although Cu(III) has yet to be observed in a metalloenzyme, Cu(III)$O_2$ complexes are common in synthetic chemistry (39) suggesting that it may be biologically possible. Residues for the di-copper site model, however, are not found in some pMMO sequences, e.g., verrucomicrobial pMMOs (40), and acetylene, a well-known suicide inhibitor of pMMO labels PmoA, not PmoB (41), suggesting that methane oxidation may not occur at this site. Another model proposes that the active site in pMMO is a tricopper Cu(II)Cu(II)($\mu$-O)$_2$Cu(III) complex site coordinated by residues from PmoA and PmoC (the $\beta$- and $\gamma$-subunits, respectively [42]). In support of this hypothesis, the proponents of this model show that model tricopper complexes can oxidize methane to methanol (43). A third model, based on Mössbauer spectroscopy coupled with activity measurements and metal analyses of purified pMMO, suggests that a di-iron site, similar to that found in sMMO and coordinated by residues from PmoA and PmoC, is responsible for methane oxidation (44). In support of this argument, it has been found that mutations of the predicted ligands of this site in the very similar membrane-bound hydrocarbon monooxygenase of *Mycobacterium* NBB4 abolished its activity (40).

Despite the dispute as to the exact composition of the active site of pMMO, it is generally agreed that copper is an essential component of the enzyme, as there is an additional metal-binding site in PmoB that contains a single copper (45–52). In fact, some evidence suggests that this may play an important role in methane oxidation, e.g., DFT and QM/MM studies indicate that dioxygen could be incorporated with Cu(I) to ultimately create a Cu(III)-oxo species that could convert methane to methanol (53). Further, single copper sites have been shown capable of oxidatively cleaving glycosidic bonds (54, 55), suggesting that a similar mechanism could be used to oxidize methane.

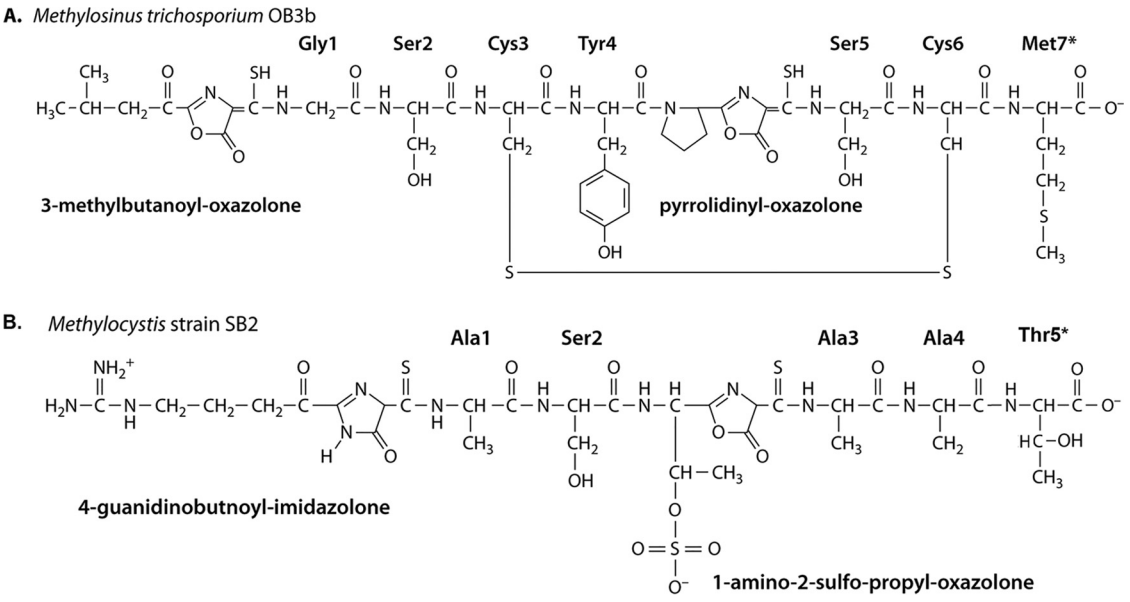


**FIG 3** Representative primary structures of group I (A) and group II (B) MBs. MBs from *M. trichosporium* OB3b (A) and *Methylocystis* strain SB2 (B) are shown (65, 166). *, Met7 and Thr5 are absent in a fraction of MB from *M. trichosporium* OB3b and *Methylocystis* strain SB2, respectively (59, 167). Loss of these amino acids does not change metal-binding affinity.

Given the potential widespread industrial application of a biomimetic catalyst capable of converting methane to methanol under ambient pressures and temperatures, there is a great deal of interest in delineating how pMMO oxidizes methane. It is clear, however, that more work must be pursued in characterizing this intriguing enzyme to achieve that goal.

**Characterization of methanobactin, a novel copper binding molecule, or chalkophore.** Methanotrophs clearly respond to copper, and as such, these microbes must have some mechanism to sense and collect copper from the environment. Initial evidence for such machinery came from the characterization of constitutive sMMO mutants (sMMO$^{C}$) of *Methylosinus trichosporium* OB3b constructed using random chemical mutagenesis (56, 57). These mutants had impaired copper uptake compared to the *M. trichosporium* OB3b wild type, suggesting that methanotrophs can synthesize a copper-complexing agent, or chalkophore ("chalko" is Greek for "copper"). Subsequently, this chalkophore was isolated, purified, and characterized from *M. trichosporium* OB3b (58). This chalkophore, called methanobactin (MB), was found to be a modified polypeptide containing two oxazolone rings with associated thioamide groups that collectively are responsible for copper binding with extremely high affinity (reported binding constants range from $10^{18}$ to $10^{58}$ $M^{-1}$ and vary depending on the solution conditions and method used to determine affinity [59–64]). Subsequently, MBs have been characterized from five other methanotrophs, all grouping in the *Alphaproteobacteria* (59, 65, 66). All these forms are small (<1,300 Da) but can be divided into two general groups (Fig. 3). Group I MBs have two oxazolone groups, while those in group II have a C-terminal oxazolone ring, with the other ring being either an imidazolone or a pyrazinedione moiety. Group I MBs contain Cys residues in the mature peptide, while those in group II do not. Additionally, a sulfate group is not found in group I MBs but is present in all structurally characterized group II MBs (59, 64, 65).

**Biosynthesis and genetics of methanobactin.** Given the unique structure of MB, it was initially speculated that it was synthesized via a nonribosomal peptide synthase (67). However, our subsequent work showed that it was a ribosomally synthesized and posttranslationally modified peptide, with the gene encoding the precursor polypeptide, *mbnA*, part of a cluster that includes genes either known or suspected to be involved in MB regulation, synthesis, secretion or uptake (Fig. 4). Upstream of *mbnA* is

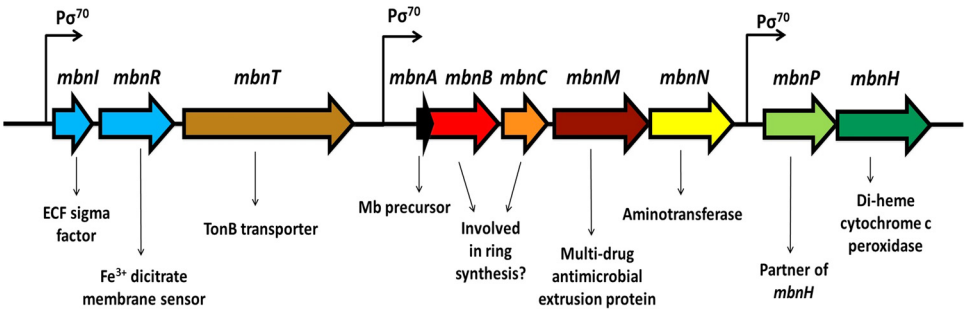


**FIG 4** Methanobactin gene cluster from *M. trichosporium* OB3b. ECF, extracytoplasmic function.

*mbnT*, encoding a TonB-dependent transporter that we have shown is responsible for MB uptake (68). Further upstream are *mbnR* and *mbnI*, encoding a putative membrane sensor and an extracytoplasmic function sigma factor, respectively, that might play a regulatory role in MB biosynthesis. Downstream of *mbnA* in *M. trichosporium* OB3b are several other genes (*mbnBCMNPH*) that are known or suspected to be involved in the biosynthesis of MB from the precursor peptide or in MB secretion.

The sequence of *mbnB* suggests both an *S*-adenosyl-L-methionine-dependent methyltransferase and a triphosphate isomerase (TIM)-barrel domain (as predicted by Phyre2 [69]). This suggests that MbnB may have lyase, hydrolase, methyltransferase, and/or isomerase activity (70, 71). The sequence of *mbnC* includes the ligase domain of phosphoribosylformylglycinamidine synthase, as well as a flavodoxin-like fold (72). Consistent with this homology, *mbnC* may be required for the formation of one or both of the heterocyclic rings. *mbnM* encodes a multidrug and toxin extrusion (MATE) protein and may be responsible for MB secretion (64, 65, 73, 74). *mbnN* is annotated as an aminotransferase that we have shown is critical for the formation of one of the two oxazolone rings in MB of *M. trichosporium* OB3b (75). *mbnH* encodes a MauG type di-heme cytochrome *c* peroxidase. In methylotrophic bacteria capable of growth on methylamine, MauG is required for the oxidative modification of two tryptophans into the cofactor tryptophan tryptophylquinone found in methylamine dehydrogenase (76–80). No methylamine dehydrogenase-like gene is present in the *M. trichosporium* OB3b genome (data not shown). Given this and the location of MbnH in the MB-OB3b operon, it appears that MbnH may be involved in oxidative step(s) required for ring formation (64). Its partner protein, MbnP, is a metallo-mystery 4-Cys motif protein.

It is difficult to predict using *mbnA* sequences if any particular microbe has the potential to produce MB; rather, interrogation of genomes for *mbnBC* is much more informative. Using these genes as markers (although, to date, only five forms of MB have been structurally characterized), bioinformatic analyses indicate that other methanotrophs in the *Alphaproteobacteria* as well as some nonmethanotrophs have genes for MB biosynthesis (Table 1) (65, 73, 74, 81). Such findings suggest that MB production may be more extensive than currently known.

**Genetic basis for the copper switch.** The *mmo* operon is composed of six genes, *mmoXYBZDC*. Of these genes, *mmoXYBZC* are known to encode peptides of hydroxylase, reductase, and regulatory protein, but the function of *mmoD* was unknown for many years. Characterization of the sMMO minus deletion mutant (SMDM), where the *mmoXYBZDC* genes were knocked out in *M. trichosporium* OB3b (82), found that the copper switch was inverted compared to the wild type (74). It was thus proposed that MmoD plays a critical role in the copper switch. This conclusion was further supported by others that found an *mmoD* deletion mutant in *Methylomicrobium buryatense* had no sMMO activity in the absence of copper (83). It should be stressed, however, that although MmoD plays an important role in the copper switch, it is likely that additional elements exist (64, 74). For example, it appears that MB serves to amplify the magnitude of the copper switch (74). Further, two genes, *mmoR* and *mmoG*, have been shown to play key roles in the regulation of expression of the *mmo* operon and that some sort

of complex may be formed between the products of these genes with methanobactin and/or MmoD. It is also possible that the putative regulatory genes associated with methanobactin, *mbnI* and *mbnR*, may play a role in the copper switch. These hypotheses must be treated cautiously, however, as no experimental data have been presented to support them.

**Whole-cell transcriptome response to copper.** Recently, the transcriptome of *M. trichosporium* OB3b was characterized in the presence and absence of copper with the objective of determining how broadly copper affected gene expression in methanotrophs, and of identifying additional elements of the copper switch (84). When *M. trichosporium* OB3b was grown in the presence of 10 $\mu$M copper, approximately 100 genes were found to be either significantly up- or downregulated compared to when no copper was added. As expected, this included genes encoding polypeptides of sMMO and pMMO, as well as several putative transcriptional regulators that were significantly repressed in the presence of copper. We speculate that these putative regulatory elements may be involved in the copper switch, but this has yet to be shown.

Other genes were also found to be differentially expressed with respect to copper, including many genes involved in copper homeostasis, indicating that methanotrophs must carefully control copper speciation and distribution to limit the toxicity of copper. For example, it was found that in *M. trichosporium* OB3b, the expression of genes involved in MB transport and synthesis were repressed when copper was present (84). Additionally, two genes encoding recently identified copper storage proteins, Csp1 and Csp2 (85, 86), as well as *cusA*, encoding a copper efflux system (87), were significantly upregulated in the presence of copper. Such findings indicate careful regulation of copper uptake by *M. trichosporium* OB3b (84). Similar results have been reported for *M. capsulatus* Bath, with differential expression of *c*-type cytochromes associated with the cell surface observed, suggesting that these may play a role in copper homeostasis for this methanotroph (88, 89). Thus, although copper is very important in methanotrophic metabolism, its speciation and distribution must be carefully controlled to limit its inherent toxicity, e.g., its high redox activity and binding to iron-sulfur cluster sites (90, 91).

## IMPLICATIONS OF METHANOBACTIN-METAL INTERACTIONS

**Microbial competition for copper is regulated by methanobactin.** Given that methanobactin binds copper with extraordinarily high affinity, Chang et al. (92) hypothesized that methanotrophs, through the production of methanobactin, could effectively "starve" other microbes for copper. Specifically, denitrifying microbes also require substantial amounts of copper for the activity of the nitrous oxide reductase NosZ (93). If this hypothesis is correct, denitrifiers when incubated in the presence of MB would not completely reduce nitrate to dinitrogen; rather, nitrous oxide would be the terminal product. Indeed, when axenic cultures of the denitrifier *Pseudomonas stutzeri* DCP-Ps1 were examined, very small and transient amounts of $N_2O$ were observed (~0.01% of added $NO_3^-$). When *P. stutzeri* DCP-Ps1 was incubated either in the presence of *M. trichosporium* OB3b or purified MB from this methanotroph (MB-OB3b), all added $NO_3^-$ was converted to $N_2O$, with no further reduction (92). Further, when *P. stutzeri* DCP-Ps1 was incubated in the presence of a mutant of *M. trichosporium* OB3b incapable of expressing MB, $N_2O$ production trends were identical to those of axenic cultures of *P. stutzeri* DCP-Ps1. Similar findings were found with three other denitrifiers that express NosZ (92). As methanotrophs and denitrifiers can and do spatially overlap in the environment, these findings indicate that competition for copper may significantly affect net greenhouse gas emissions *in situ*. That is, the substrates for aerobic methanotrophs are $CH_4$ and $O_2$ that diffuse toward the oxic-anoxic interface from opposite ends of the oxygen gradient. As a result, the largest abundance of methanotrophs is often found at the oxic-anoxic interfaces in subsurface environments (94, 95) that are also often a "hot spot" for soil denitrification (96).

In addition, copper competition between methanotrophs and other microbes may limit the activity of other copper-dependent enzymes, e.g., the nitrite reductase NirK and ammonia monooxygenase (93, 97). Indeed, it has been found that methanobactin can inhibit the activity of NirK in *Shewanella loihica* (92). Additional work in this area is clearly warranted.

**Methanobactin can bind and detoxify mercury.** Methanobactin from *M. trichosporium* OB3b (MB-OB3b) and *Methylocystis* strain SB2 (MB-SB2) will bind, in addition to Cu(II), Hg(II), and do so irreversibly, even in the presence of copper (98, 99). Such irreversible binding appears to be due to the rapid binding of Hg(II), i.e., when we attempted to measure the kinetics of Hg(II) binding by MB-OB3b, over 90% of the Hg(II) added was bound during the 1.8-ms dead time of the stopped flow reactor, and the remaining 10% had an observed binding rate of $640 \pm 43 \cdot s^{-1}$ (98). Not only was Hg(II) found to be quickly and irreversibly bound by MB-OB3b, it substantially reduced the toxicity of Hg(II) to *M. trichosporium* OB3b, as well as to other methanotrophs; i.e., methanobactin can act as a general prophylactic and protect the broader microbial community from the toxic effects of mercury. In the case of MB-SB2, Hg(II) binding rates were $>2{,}000 \cdot s^{-1}$, and Hg(II) could displace Cu(I) from the MB-SB2-Cu complex (99).

We have also shown methanobactin can bind methylmercury (99), suggesting that methanobactin can also control its bioavailability. Indeed, we have found that *M. trichosporium* OB3b takes up and degrades methylmercury (100). This is surprising, as *merB*, encoding organomercurial lyase, is not present in the genome of *M. trichosporium* OB3b, indicating that this strain uses another mechanism to demethylate methylmercury. It should also be noted that *M. trichosporium* OB3b degraded methylmercury at concentrations commonly found in the environment, e.g., nanomolar concentrations (100). MerB has a much weaker affinity for methylmercury ($K_m$ of 500 $\mu$M) (101), and as a result, methanotroph-mediated methylmercury degradation may have great environmental significance.

Not all methanotrophs, however, can degrade methylmercury. Specifically, *Methylococcus capsulatus* Bath was found to sorb substantial amounts of methylmercury, but it did not degrade it. Genes encoding methanobactin biosynthesis are not found in *M. capsulatus* Bath. Rather, *M. capsulatus* Bath relies on a surface-bound protein with a tryptophan converted to a kynurenine (MopE) and a secreted form of it (MopE*) for copper binding (102–105). It thus appears that methylmercury degradation by methanotrophs requires that the cells first express methanobactin. This hypothesis was tested by examining methylmercury degradation by *Methylocystis* strain SB2 (capable of expressing MB) as well as two mutant strains of *M. trichosporium* OB3b defective in methanobactin production. *Methylocystis* strain SB2 degraded methylmercury, whereas MB-defective strains did not, showing that MB is required for methanotrophic-mediated methylmercury degradation. Methanobactin, although necessary, is not sufficient for methylmercury degradation by methanotrophs. When methylmercury was incubated with purified MB-OB3b, no appreciable methylmercury degradation was observed (100). This suggests that methanobactin served as a means of bringing methylmercury into the cell, where it is degraded by an as-yet-unknown mechanism.

## FUNCTION OF RARE-EARTH ELEMENTS IN METHANOTROPHS

It is well known that microbial physiology requires a broad suite of metals, but some metals historically have been viewed as having little if any biological relevance. Prime examples of such metals are the rare-earth elements. These metals, despite being characterized as rare, are actually quite abundant in Earth's crust, e.g., the average abundance of cerium is 66 $\mu g \cdot g^{-1}$, similar to that for zinc and copper (106). These elements, however, are typically found in extremely insoluble forms, e.g., oxides, sulfides, carbonates, and phosphates (107). Given their poor solubility, the lack of any identified biological mechanisms for uptake, and the fact that they had no established biological function, it was commonly presumed that these elements were nonessential. Despite this prevailing dogma, studies from as early as 1961 showed that some microbes could sequester large amounts of some of these metals (108). Further, it was speculated over

10 years ago that enzymes containing rare-earth elements would be catalytically superior to enzymes containing chemically similar calcium, as rare-earth elements are much stronger Lewis acids than calcium and thus are likely much more efficient catalysts for hydrolysis (109).

**REEs and methanol dehydrogenase.** Methanol oxidation is carried out by methanol dehydrogenase (MeDH). Initial characterization of MeDH found it to be a heterotetramer composed of two subunits, the large subunit MxaF (65 kDa) and the small subunit MxaI (8.5 kDa). MxaF contains pyrroloquinoline quinone (PQQ) and $Ca^{2+}$ as catalytic cofactors (110–113). Subsequently, a homolog to *mxaF*, termed *xoxF*, was found in the methylotrophs *Paracoccus denitrificans* and *Methylobacterium extorquens* AM1 (114, 115). A sequence comparison revealed that most amino acid residues involved in PQQ and calcium binding in *mxaF* were conserved in *xoxF*, indicating that it was likely involved in methanol oxidation (114, 116). Early mutagenesis studies of *xoxF* in methylotrophs, however, showed either no effect or some reduction in growth on methanol (115, 116), presumably due to the coexistence of *mxaF* in the microbes examined. The deletion of *xoxF* in *Rhodobacter sphaeroides*, however, caused the resulting mutant to be unable to grow on methanol, as *R. sphaeroides* does not have *mxaF*, showing that Xox-MeDH is a true MeDH (117).

Despite this clear finding, characterization of Xox-MeDH was initially quite challenging, as its expression was typically very low, and active preparations were difficult, if not impossible, to create (118, 119). A critical breakthrough in the characterization of Xox-MeDH came from the observation that its activity was dependent on the presence of REEs (120–123). Specifically, Xox-MeDH activity increased ~5- to 10-fold with addition of lanthanum in the growth medium of the methylotrophs *Methylobacterium radiotolerans* and *Methylobacterium extorquens* AM1 (120, 122). Further, purified Xox-MeDH from *M. extorquens* AM1 was found to predominantly contain lanthanum (122). Subsequently, it was discovered that growth of the acidophilic methanotroph *Methylacidiphilum fumariolicum* SolV, which can express Xox-MeDH but not Mxa-MeDH, was strictly dependent on the addition of REEs, with growth stimulated the greatest with "light" REEs (i.e., lanthanum, cerium, praseodymium, and neodymium). As found in *M. extorquens* AM1, purified Xox-MeDH from *M. fumariolicum* SolV also contained a rare-earth element (123).

In rapid succession, a number of papers documented that the expression of Mxa-MeDH and Xox-MeDH in a variety of methanotrophs and methylotrophs was strongly dependent on the availability of REEs, primarily light REEs (124–128). In the absence of REEs, little expression of *xox* genes was observed, while the expression of *mxa* genes was high. In the presence of REEs, however, the expression of *xox* genes increased significantly, while the expression of *mxa* genes decreased. That is, in addition to the copper switch controlling the expression of pMMO/sMMO, there is an "REE switch" regulating the expression of Mxa-MeDH/Xox-MeDH. These switches appear to have some overlap, with the copper switch capable of "overriding" part of the REE switch in at least one methanotroph. Specifically, when *M. trichosporium* OB3b was grown in the presence of copper, little repression of *mxaF* expression by REEs was observed, although substantial (>2 orders of magnitude) repression was observed when REEs were added in the absence of copper. The expression of *xoxF*, however, was not significantly different when REEs were added in the absence or presence of copper (125).

**Microbial uptake of REEs.** Although REEs clearly are taken up (as evidenced by the response of many methanotrophs and methylotrophs to these metals), the mechanism(s) is (are) unknown. Such uptake is particularly challenging, for as noted earlier, REEs are noted by their very low dissolution in water, e.g., $CePO_4$ has a solubility of $10^{-23}$ g per 100 g of water (129). Nonetheless, there are several possibilities as to how REEs are collected: (i) adventitious leaching of REEs via secretion of low-molecular-weight organic acids, inorganic acids, and/or metal-binding compounds, such as siderophores or chalkophores that dissolve cerium-containing minerals (130–137); (ii) secretion of a specific REE-binding compound; and/or (iii) uptake of rare-earth elements

as REE-phosphates via systems, such as the $P_i$ transport system (Pit). This low-affinity high-velocity system is used by many microbes for phosphate uptake and has been shown to control the accumulation of metals, such as zinc (138–140). None of these possibilities can be excluded at this time. Methanotrophs are known to produce both chalkophores and siderophores (141), and these may be partially responsible for increasing the bioavailability of cerium. Further, it was discovered over 40 years ago that methanotrophs produce a number of water-soluble pigments that may include novel metal-binding compounds (142). Methanotrophs have also been shown to produce low-molecular-weight organic acids under microaerobic conditions (143), suggesting that this may play a role in the leaching of rare-earth elements. Finally, a preliminary review of the genome of *M. trichosporium* OB3b indicates that this strain does indeed have the $P_i$ transport system (data not shown). It may be that one system or some combination of these systems is used for rare-earth element collection by methanotrophs. As REEs are difficult to purify, elucidation of the mechanism by which methanotrophs take up rare-earth elements may have significant industrial application (144).

**Genetic basis of the REE switch.** There is a great deal yet to be learned about the basis of the REE switch, but work from the University of Washington has begun to address this issue, i.e., Chu et al. (145) found that a histidine kinase, MxaY, is a key part of the REE switch. The deletion of *mxaY* abolishes the response of *mxaF* and *xoxF* in *M. buryatense* to lanthanum. It further appears that MxaY controls the expression of the *mxa* and *xox* genes by at least partially controlling expression of another response regulator, MxaB, although it is still unknown if MxaY and MxaB directly interact or if there are other components to the REE switch.

To identify what, if any, other elements make up the REE switch, the transcriptome of *M. trichosporium* OB3b was characterized in the presence and absence of cerium (84). Using stringent cutoff criteria of a $|\log_2|$-fold change of $>1.5$ and a Benjamini-Hochberg-adjusted *P* value of $<1 \times 10^{-3}$, one putative sigma factor was found to be upregulated in the presence of cerium, suggesting that it may also play a part in the REE switch (84). Interestingly, when using these strict criteria, *mxaY* and *mxaB* expression was not found to vary with the presence of cerium. If, however, a more relaxed (but still stringent) criteria of a $|\log_2|$-fold change of $>1$ and a Benjamini-Hochberg-adjusted *P* value of $<1 \times 10^{-3}$ were used, *mxaY* and *mxaB* expression was found to be repressed when cerium was added, supporting the conclusion of Chu et al. (145).

Interestingly, although the expression of Mxa-MeDH and Xox-MeDH in methanotrophs is strongly controlled by the availability of REEs, it appears that the REE switch is limited to these forms of MeDH. That is, in a comparison of the transcriptome of *M. trichosporium* OB3b grown in the presence of 25 $\mu$M cerium versus 0 $\mu$M cerium, the expression of only a small number of genes not involved in either Mxa-MeDH or Xox-MeDH had differential expression (84). Perhaps most interesting was that the expression of genes involved in the conversion of formaldehyde to formate via the tetrahydromethanopterin and tetrahydrofolate pathways was not affected by the presence or absence of cerium. Such a finding is intriguing, as it has been shown that Xox-MeDH not only can bind methanol with very high affinity, it can also oxidize formaldehyde, indicating that Xox-MeDH transforms methanol directly to formate (146). For methanotrophs that rely on the CBB cycle for carbon fixation, such a phenomenon is of little consequence, as formate can be oxidized further to $CO_2$. Methanotrophs that rely on the ribulose monophosphate (RuMP) or serine cycle for carbon assimilation, however, must produce formaldehyde for growth. For example, methanotrophs, such as *M. trichosporium* OB3b, that utilize the serine cycle convert formaldehyde to methylene tetrahydrofolate that is then inserted into the serine cycle. Methylene tetrahydrofolate, however, can also be formed via the condensation of formate with tetrahydrofolate, followed by subsequent reduction to methylene tetrahydrofolate (Fig. 2). In this pathway, formate is first converted to formyl-tetrahydrofolate via the formate tetrahydrofolate ligase (Ftfl). Formyl-tetrahydrofolate is then reduced to methylene tetrahydrofolate via a two-step process mediated by methenyltetrahydrofolate cyclohydrolase (FchA) and methylene

tetrahydrofolate dehydrogenase (MtdA). Genes encoding all of these steps (*ftfl*, *fchA*, and *mtdA*), however, were not upregulated when *M. trichosporium* OB3b was grown in the presence of cerium (84), suggesting that either these enzymes are highly active or that formate is not the primary product of methanol oxidation by Xox-MeDH in *M. trichosporium* OB3b. It has been suggested that Xox-MeDH may, depending on the microbe, release formaldehyde rather than formate (146). Probing the metabolome of *M. trichosporium* OB3b and other methanotrophs that rely on either the RuMP or serine pathway and that are solely expressing Xox-MeDH is likely to be very informative in resolving the product(s) of Xox-MeDH *in vivo* for these methanotrophs.

**Environmental significance of Xox-methanol dehydrogenase.** Recently, it has been shown that not only are genes encoding Xox-MeDH common to many environmental samples (e.g., see references 147–153), they exhibit much greater diversity than Mxa-MeDHs (146). Such findings mean that REE-containing MeDHs might in fact be more environmentally relevant than the "classical" or earlier-characterized Mxa-MeDH. Indeed, methanotrophs have been found to possess only Xox-MDH and not Mxa-MDH (Table 1) (154–158). Further, recent findings show that not only is *xoxF* widespread in marine environments (147), it is one of the most abundant transcripts found in marine methylotrophs (159). In addition, dissolved amounts of light REEs substantially decreased in the methane plume associated with the Deepwater Horizon blowout of 2010, and methane consumption correlated with light-REE depletion (160). Collectively, these data strongly imply that Xox-MeDH may be the predominant form of MeDH expressed *in situ*, suggesting that it confers a selective advantage over the Mxa-MeDH for growth on $C_1$ compounds. Indeed, it has been shown that when expressing Xox-MeDH, the methanotroph *Methylobacter tundripaludum* did not excrete methanol, but it did to a level of 1.24 mM when Mxa-MeDH was expressed, likely due to the poorer affinity for methanol exhibited by the Mxa-MeDH (161). As such, the expression of Xox-MeDH could improve methanotrophic growth yield and/or carbon conversion efficiency by limiting the loss of methanol.

## WHAT ARE THE ENVIRONMENTAL ROLES OF THE COPPER AND REE SWITCHES?

Although the copper switch is evident in the laboratory, it is less clear what benefit(s) it might provide for methanotrophic growth *in situ*. One can speculate that when methane is plentiful, methanotrophic biomass will increase, reducing the overall copper-to-biomass ratio, thereby inducing the expression of sMMO, which has a higher turnover rate of methane but weaker affinity than pMMO (27). Conversely, if methane concentrations are low, methanotrophic growth would be expected to be reduced, resulting in diminished overall biomass. Methanotrophs that express the copper switch, however, may continue to predominate by expressing pMMO under these conditions. A question, however, that has been largely ignored in the scientific literature is why do so few known methanotrophs have the copper switch, particularly if one accepts the hypothesis that it provides greater flexibility for these methanotrophs to thrive under varied methane availabilities? This cannot be unequivocally answered at this time, but it may be that the copper switch comes at some fitness cost, preventing methanotrophs exhibiting it from effectively competing with other methanotrophs that only express one form of MMO when methane concentrations are at the extremes (i.e., very low or very high).

For the REE switch, as mentioned earlier, there is clear evidence that the expression of Xox-MeDH can be advantageous to methanotrophs, as it can limit the loss of methanol (161). As such, it would appear that Xox-MeDH expression *in situ* would provide methanotrophs with a competitive advantage, especially as REEs are common, as noted earlier (106). However, in simple two-member communities where a methanotroph (*Methylobacter tundripaludum*) was incubated in the presence of a methylotroph (either *Methylotenera mobilis* 13 or *M. mobilis* JW8) with methane provided as the sole carbon source and in the presence of 30 $\mu$M lanthanum (as a representative REE), Mxa-MeDH expression by *M. tundripaludum* increased and Xox-MeDH expression decreased compared to when *Methylobacter tundripaludum* was grown axenically in the

presence of lanthanum (161). It appears that the methylotroph, through the secretion of some soluble compound(s), either limited REE uptake by methanotrophs, or that this compound(s) acted as some signal to override the REE switch. Clearly, in these systems, it is beneficial for the methylotroph to induce the expression of Mxa-MeDH in the methanotrophs to increase methanol secretion and allow cross-feeding to occur. What is less clear is what if any benefit does the methanotroph receive in kind? It has been reported that methane oxidation by a single methanotroph (*Methylomonas methanica*) increased in artificial mixed communities with increasing heterotroph richness (162), suggesting that the heterotrophs provide some metabolite(s) that promotes methanotrophic growth, e.g., perhaps cobalamin that can stimulate methanotrophic growth (163). It may be that having the flexibility to express either form of MeDH enables methanotrophs to more positively interact with important microbial partners to the benefit of all.

## CONCLUSIONS

Methanotrophy continues to offer up surprises, and the recent findings of multiple metal switches in these intriguing microbes provide new opportunities to manipulate these microbes for a variety of opportunities. Despite our expanded knowledge on metal-methanotroph interactions, there is still much we do not know, including the following questions.

**What is the complete basis of both the copper switch and REE switch in methanotrophs?** Such information could be generated via selective knockouts of suspected regulatory genes identified via transcriptomic analyses. Can we then manipulate these switches in some way to enhance methane valorization? Elucidation of these switches could also be of use in synthetic biology by expanding the "toolbox" available for the construction of robust genetic circuits with unique input parameters.

**What genes are required for methanobactin biosynthesis from a precursor polypeptide?** A two-pronged approach could be used here. First, methanotrophic mutants could be created where individual genes suspected to be involved in MB biosynthesis are knocked out, followed by characterization of MB intermediates made by these mutants. With such information, the biosynthetic pathway of MB could be reconstructed, and indeed, work has begun using this approach (75). Second, heterologous expression of MB could be attempted where various combinations of genes known or suspected to be involved in MB biosynthesis are inserted in hosts, such as *Escherichia coli*.

**How is methylmercury degraded by methanotrophs expressing methanobactin?** Stable isotope labeling of methylmercury to follow products of methylmercury degradation would be very useful here, as well as knockouts of gene(s) suspected to be involved. Once the mechanism of methanotrophic-mediated methylmercury degradation is identified, this could then be used as a signature to determine how prevalent such an ability is *in situ*.

**How widespread is copper competition *in situ*?** How does this impact the emissions of not only methane but also nitrous oxide? Future studies should consider a combination of simple soil microcosm studies where methanotrophs and denitrifiers are cocultured to delineate methanotroph-denitrifier competition for copper, as well as more complex field studies where activities of these microbes are carefully monitored and correlated to net emissions of both methane and nitrous oxide.

**How do methanotrophs collect REEs that most commonly are found in extremely insoluble forms?** As noted, there are several possibilities, but at this moment, it is still unclear what the mechanism(s) may be. One strategy to begin identifying the mechanism could be to incubate methanotrophs in the presence of different dissolved concentrations of REEs, e.g., REEs in the presence/absence of chelating agents, such as nitrilotriacetic acid, and then compare metal uptake as well as the transcriptome/proteome of these cultures. Doing so may generate clues as to the putative REE uptake system. If this is successful, it may be possible to use this information to enhance the extraction/purification of REEs.

Answering these questions, although likely to be challenging, is also likely to be of great value.

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## ADDENDUM IN PROOF

After revising the manuscript, L. Cao, O. Caldararu, A. C. Rosenzweig, and U. Ryde published an article (Angew Chem Int Ed 57:162–166, 2018, https://doi.org/10.1002/anie.201708977) concluding, using quantum refinement studies, that the proposed di-copper active site of pMMO (36) does not exist in the crystal structure of this enzyme. Rather, this site is best modeled as a mono-copper site. As such, we believe it important to reiterate that much more work is required to understand the mechanism of methane oxidation by pMMO.

## REFERENCES

1. Kalyuzhnaya MG, Puri AW, Lidstrom ME. 2015. Metabolic engineering in methanotrophic bacteria. Metab Eng 29:142–152. https://doi.org/10.1016/j.ymben.2015.03.010.
2. Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, Jetten MSM, Birkeland NK, Pol A, Dunfield PF. 2009. Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environ Microbiol Rep 1:293–306. https://doi.org/10.1111/j.1758-2229.2009.00022.x.
3. Strong PJ, Xie S, Clarke WP. 2015. Methane as a resource: can the methanotrophs add value? Environ Sci Technol 49:4001–4018. https://doi.org/10.1021/es504242n.
4. Strong PJ, Kalyuzhnaya M, Silverman J, Clarke WP. 2016. A methanotroph-based biorefinery: potential scenarios for generating multiple products from a single fermentation. Bioresour Technol 215:314–323. https://doi.org/10.1016/j.biortech.2016.04.099.
5. Semrau JD, DiSpirito AA, Yoon S. 2010. Methanotrophs and copper. FEMS Microbiol Rev 34:496–531. https://doi.org/10.1111/j.1574-6976.2010.00212.x.
6. Knief C. 2015. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. Front Microbiol 6:1346. https://doi.org/10.3389/fmicb.2015.01346.
7. Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S, Ly B, Saw JH, Zhou Z, Ren Y, Wang J, Mountain BW, Crowe MA, Weatherby TM, Bodelier PL, Liesack W, Feng L, Wang L, Alam M. 2007. Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882. https://doi.org/10.1038/nature06411.
8. Anvar SY, Frank J, Pol A, Schmitz A, Kraaijeveld K, den Dunnen JT, Op den Camp HJ. 2014. The genomic landscape of verrucomicrobial methanotroph *Methylacidiphilum fumariolicum* SolV. BMC Genomics 15:914–926. https://doi.org/10.1186/1471-2164-15-914.
9. Bowman JP, McCammon SA, Skerratt JH. 1997. *Methylosphaera hansonii* gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic marine-salinity, meromictic lakes. Microbiology 143:1451–1459. https://doi.org/10.1099/00221287-143-4-1451.
10. Kaluzhnaya M, Khmelenina V, Eshinimaev B, Suzina N, Nikitin D, Solonin A, Lin JL, McDonald I, Murrell C, Trotsenko Y. 2001. Taxonomic characterization of new alkaliphilic and alkalitolerant methanotrophs from soda lakes of the Southeastern Transbaikal region and description of *Methylomicrobium buryatense* sp. nov. Syst Appl Microbiol 24:166–176. https://doi.org/10.1078/0723-2020-00028.
11. Islam T, Jensen S, Reigstad LJ, Larsen Ø, Birkeland N-K. 2008. Methane oxidation at 55°C and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. Proc Natl Acad Sci U S A 105:300–304. https://doi.org/10.1073/pnas.0704162105.
12. Omelchenko MV, Vasilyeva LV, Zavarzin GA. 1993. Psychrophilic methanotroph from tundra soil. Curr Microbiol 27:255–259. https://doi.org/10.1007/BF01575988.
13. Omel'chenko MV, Vasil'eva LV, Zavarzin GA, Savel'eva ND, Lysenko AM, Mityushina LL, Khmelenina VN, Trotsenko YA. 1996. A novel psychrophilic methanotroph of the genus *Methylobacter*. Microbiology 65:339–343.
14. Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, Op den Camp HJM. 2007. Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature 450:874–818. https://doi.org/10.1038/nature06222.
15. Doronina NV, Ivanova EG, Suzina NE, Trotsenko YA. 2004. Methanotrophs and methylobacteria are found in woody plant tissues within the winter period. Microbiology 73:702–709. https://doi.org/10.1007/s11021-005-0012-0.
16. Iguchi H, Sato I, Sakakibara M, Yurimoto H, Sakai Y. 2012. Distribution of methanotrophs in the phyllosphere. Biosci Biotechnol Biochem 76:1580–1583. https://doi.org/10.1271/bbb.120281.
17. Iguchi H, Sato I, Yurimoto H, Sakai Y. 2013. Stress resistance and $C_1$ metabolism involved in plant colonization of a methanotroph *Methylosinus* sp. B4S. Arch Microbiol 195:717–726. https://doi.org/10.1007/s00203-013-0922-6.
18. Yoshida N, Iguchi H, Yurimoto H, Murakami A, Sakai Y. 2014. Aquatic plant surface as a niche for methanotrophs. Front Microbiol 5:30. https://doi.org/10.3389/fmicb.2014.00030.
19. van Teeseling MCF, Pol A, Harhangi HR, van der Zwart S, Jetten MSM, Op den Camp HJM, van Niftrik L. 2014. Expanding the verrucomicrobial methanotrophic world: description of three novel species of *Methylacidimicrobium* gen. nov. Appl Environ Microbiol 80:6782–6791. https://doi.org/10.1128/AEM.01838-14.
20. Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MMM, Schreiber F, Dutilh BE, Zedelius J, De Beer D, Gloerich J, Wessels HJ, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJ, Janssen-Megens EM, Francoijs KJ, Stunnenberg H, Weissenbach J, Jetten MS, Strous M. 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548. https://doi.org/10.1038/nature08883.
21. Rasigraf O, Kool DM, Jetten MSM, Sinninghe Damsté JS, Ettwig KF. 2014. Autotrophic carbon dioxide fixation via the Calvin-Benson-Bassham cycle by the denitrifying methanotroph "*Candidatus* Methylomirabilis oxyfera." Appl Environ Microbiol 80:2451–2460. https://doi.org/10.1128/AEM.04199-13.
22. Khadem AF, Pol A, Wieczorek A, Mohammadi SS, Francoijs KJ, Stunnenberg HG, Jetten MSM, Op den Camp HJM. 2011. Autotrophic methanotrophy in *Verrucomicrobia*: *Methylacidiphilum fumariolicum* SolV uses the Calvin-Benson-Bassham cycle for carbon dioxide fixation. J Bacteriol 193:4438–4446. https://doi.org/10.1128/JB.00407-11.
23. Ward N, Larsen O, James S, Bruseth L, Khouri H, Durkin AS, Dimitrov G, Jiang L, Scanlan D, Kang KH, Lewis M, Nelson KE, Methe' B, Wu M,

Heidelberg JE, Paulsen IT, Fouts D, Ravel J, Tettelin H, Ren Q, Read T, DeBoy RT, Seshadri R, Salzberg SL, Jensen HB, Birkeland NK, Nelson WC, Dodson RJ, Grindhaug SH, Holt I, Eidhammer I, Jonasen I, Vanaken S, Utterback T, Feldblyum TV, Fraser CM, Lillehaug JR, Eisen JA. 2004. Genomic insights into methanotrophy: the complete genome sequence of *Methylococcus capsulatus* (Bath). PLoS Biol 2:e303. https://doi.org/10.1371/journal.pbio.0020303.

24. Miroshnikov KK, Didriksen A, Naumoff DG, Huntemann M, Clum A, Pillay M, Palaniappan K, Varghese N, Mikhailova N, Mukherjee S, Reddy TBK, Daum C, Shapiro N, Ivanova N, Kyrpides N, Woyke T, Dedysh SN, Svenning MM. 2017. Draft genome sequence of *Methylocapsa palsarum* NE2$^T$, an obligate methanotroph from subarctic soil. Genome Announc 5(24):e00504-17. https://doi.org/10.1128/genomeA.00504-17.
25. Dedysh SN, Naumoff DG, Vorobev AV, Kyrpides N, Woyke T, Shapiro N, Crombie AT, Murrell JC, Kalyuzhnaya MG, Smirnova AV, Dunfield PF. 2016. Draft genome sequence of Methyloferula stellata AR4, an obligate methanotroph possessing only a soluble methane monooxygenase. Genome Announc 3:e01555-14. https://doi.org/10.1128/genomeA.01555-14.
26. Stanley SH, Prior SD, Leak DJ, Dalton H. 1983. Copper stress underlies the fundamental change in intracellular location of methane monooxygenase in methane-oxidizing organisms: studies in batch and continuous cultures. Biotechnol Lett 5:487–492. https://doi.org/10.1007/BF00132233.
27. Lee SW, Keeney DR, Lim DH, DiSpirito AA, Semrau JD. 2006. Mixed pollutant degradation by *Methylosinus trichosporium* OB3b expressing either soluble or particulate methane monooxygenase: can the tortoise beat the hare? Appl Environ Microbiol 72:7503–7509. https://doi.org/10.1128/AEM.01604-06.
28. Colby J, Stirling DI, Dalton H. 1977. The soluble methane monooxygenase of *Methylococcus capsulatus* (Bath). Its ability to oxygenate *n*-alkane, *n*-alkene, ethers, and alicyclic, aromatic and heterocyclic compounds. Biochem J 165:395–402.
29. Burrows KJ, Cornish A, Scott D, Higgins IJ. 1984. Substrate specificities of the soluble and particulate methane mono-oxygenases of *Methylosinus trichosporium* OB3b. J Gen Microbiol 130:3327–3333.
30. Yoon S, Semrau JD. 2008. Measurement and modeling of multiple substrate oxidation by methanotrophs at 20°C. FEMS Microbiol Lett 287:156–162. https://doi.org/10.1111/j.1574-6968.2008.01314.x.
31. Lipscomb JD. 1994. Biochemistry of the soluble methane monooxygenase. Annu Rev Microbiol 48:371–399. https://doi.org/10.1146/annurev.mi.48.100194.002103.
32. Hakemian AS, Rosenzweig AC. 2007. The biochemistry of methane oxidation. Annu Rev Biochem 76:223–241. https://doi.org/10.1146/annurev.biochem.76.061505.175355.
33. Lieberman RL, Rosenzweig AC. 2005. Crystal structure of a membrane-bound metalloenzyme that catalyses the biological oxidation of methane. Nature 434:177–182. https://doi.org/10.1038/nature03311.
34. Hakemian AS, Kondapalli KC, Telser J, Hoffman BM, Stemmler TL, Rosenzweig AC. 2008. The metal centers of particulate methane monooxygenase from *Methylosinus trichosporium* OB3b. Biochemistry 47:6793–6801. https://doi.org/10.1021/bi800598h.
35. Smith SM, Balasubramanian R, Rosenzweig AC. 2011. Metal reconstitution of particulate methane monooxygenase and heterologous expression of the *pmoB* subunit. Methods Enzymol 495:195. https://doi.org/10.1016/B978-0-12-386905-0.00013-9.
36. Balasubramanian R, Smith SM, Rawat S, Yatsunyk LA, Stemmler TL, Rosenzweig AC. 2010. Oxidation of methane by a biological dicopper centre. Nature 465:115–119. https://doi.org/10.1038/nature08992.
37. Citek C, Gary JB, Wasinger EC, Stack TDP. 2015. Chemical plausibility of Cu(III) with biological ligation in pMMO. J Am Chem Soc 137:6991–6994. https://doi.org/10.1021/jacs.5b02157.
38. Itoyama S, Doitomi K, Kamachi T, Shiota Y, Yoshizawa K. 2016. Possible peroxo state of the dicopper site of particulate methane monooxygenase from combined quantum mechanics and molecular mechanics calculations. Inorg Chem 55:2771–2775. https://doi.org/10.1021/acs.inorgchem.5b02603.
39. Mirica LM, Ottenwaelder X, Stack TDP. 2004. Structure and spectroscopy of copper-dioxygen complexes. Chem Rev 104:1013–1045. https://doi.org/10.1021/cr020632z.
40. Liew EF, Tong D, Coleman V, Holmes AJ. 2014. Mutagenesis of the hydrocarbon monooxygenase indicates a metal centre in subunit-C and not subunit-B, is essential for copper-containing membrane monooxygenase activity. Microbiology 160:1267–1277. https://doi.org/10.1099/mic.0.078584-0.
41. Prior SD, Dalton H. 1985. Acetylene as a suicide substrate and active site probe for methane monooxygenase from *Methylococcus capsulatus* (Bath). FEMS Microbiol Lett 29:105–109. https://doi.org/10.1111/j.1574-6968.1985.tb00843.x.
42. Wang VCC, Maji S, Chen PPY, Lee HK, Yu SSF, Chan SI. 2017. Alkane oxidation: methane monooxygenases, related enzymes, and their biomimetics. Chem Rev 117:8574–8621. https://doi.org/10.1021/acs.chemrev.6b00624.
43. Chen PPY, Nagababu P, Yu SSF, Chan SI. 2014. Development of the tricopper cluster as a catalyst for the efficient conversion of methane into MeOH. ChemCatChem 6:429–437. https://doi.org/10.1002/cctc.201300473.
44. Martinho M, Choi DW, DiSpirito AA, Antholine WE, Semrau JD, Münck E. 2007. Mössbauer studies of the membrane-associated methane monooxygenase from *Methylococcus capsulatus* Bath: evidence for a Diiron center. J Am Chem Soc 129:15783–15785. https://doi.org/10.1021/ja077682b.
45. Balasubramanian R, Rosenzweig AC. 2007. Structural and mechanistic insights into methane oxidation by particulate methane monooxygenase. Acc Chem Res 40:573–580. https://doi.org/10.1021/ar700004s.
46. Basu P, Katterle B, Andersson KK, Dalton H. 2003. The membrane-associated form of methane mono-oxygenase from *Methylococcus capsulatus* (Bath) is a copper/iron protein. Biochem J 369:417–427. https://doi.org/10.1042/bj20020823.
47. Choi DW, Kunz RC, Boyd ES, Semrau JD, Antholine WE, Han JI, Zahn JA, Boyd JM, de la Mora AM, DiSpirito AA. 2003. The membrane-associated methane monooxygenase (pMMO) and pMMO-NADH:quinone oxidoreductase complex from *Methylococcus capsulatus* Bath. J Bacteriol 185:5755–5764. https://doi.org/10.1128/JB.185.19.5755-5764.2003.
48. Nguyen HHT, Elliott SJ, Yip JHK, Chan SI. 1998. The particulate methane monooxygenase from *Methylococcus capsulatus* (Bath) is a novel copper-containing three-subunit enzyme. Isolation and characterization. J Biol Chem 273:7957–7966. https://doi.org/10.1074/jbc.273.14.7957.
49. Nguyen HHT, Nakagawa KH, Hedman B, Elliott SJ, Lidstrom ME, Hodgson KO, Chan SI. 1996. X-ray absorption and EPR studies on the copper ions associated with the particulate methane monooxygenase from *Methylococcus capsulatus* (Bath). Cu(I) ions and their implications. J Am Chem Soc 118:12766–12776.
50. Nguyen HHT, Shiemke AK, Jacobs SJ, Hales BJ, Lidstrom ME, Chan SI. 1994. The nature of the copper ions in the membranes containing the particulate methane monooxygenase from *Methylococcus capsulatus* (Bath). J Biol Chem 269:14995–15005.
51. Semrau JD, Zolandz D, Lindstrom ME, Chan SI. 1995. The role of copper in the pMMO of *Methylococcus capsulatus* Bath: a structural vs. catalytic function. J Inorg Biochem 58:235–244. https://doi.org/10.1016/0162-0134(94)00056-G.
52. Zahn JA, DiSpirito AA. 1996. Membrane-associated methane monooxygenase from *Methylococcus capsulatus* (Bath). J Bacteriol 178:1018–1029. https://doi.org/10.1128/jb.178.4.1018-1029.1996.
53. Yoshizawa K, Shiota Y. 2006. Conversion of methane to methanol at the mononuclear and dinuclear copper sites of particulate methane monooxygenase (pMMO): a DFT and QM/MM study. J Am Chem Soc 128:9873–9881. https://doi.org/10.1021/ja061604r.
54. Quinlan RJ, Sweeney MD, Lo Leggio L, Otten H, Poulsen JCN, Johansen KS, Krogh KBRM, Jørgensen CI, Tovborg M, Anthonsen A, Tryfona T, Walter CP, Dupree P, Xu F, Davies GJ, Walton PH. 2011. Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. Proc Natl Acad Sci U S A 108:15079–15084. https://doi.org/10.1073/pnas.1105776108.
55. Kim S, Ståhlberg J, Sandgren M, Paton RS, Beckham GT. 2014. Quantum mechanical calculations suggest that lytic polysaccharide monooxygenases use a copper-oxyl, oxygen-rebound mechanism. Proc Natl Acad Sci U S A 111:149–154. https://doi.org/10.1073/pnas.1316609111.
56. Fitch MW, Graham DW, Arnold RG, Agarwal SK, Phelps P, Speitel GE, Jr, Georgiou G. 1993. Phenotypic characterization of copper-resistant mutants of *Methylosinus trichosporium* Ob3b. Appl Environ Microbiol 59:2771–2776.
57. Phelps PA, Agarwal SK, Speitel GE, Georgiou G. 1992. *Methylosinus trichosporium* OB3b mutants having constitutive expression of soluble methane monooxygenase in the presence of high-levels of copper. Appl Environ Microbiol 58:3701–3708.

58. Kim HJ, Graham DW, DiSpirito AA, Alterman MA, Galeva N, Larive CK, Asunskis D, Sherwood PMA. 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. Science 305:1612–1615. https://doi.org/10.1126/science.1098322.
59. El Ghazouani A, Basle A, Gray J, Graham DW, Firbank SJ, Dennison C. 2012. Variations in methanobactin structure influences copper utilization by methane-oxidizing bacteria. Proc Natl Acad Sci U S A 109:8400–8404. https://doi.org/10.1073/pnas.1112921109.
60. Choi DW, Zea CJ, Do YS, Semrau JD, Antholine WE, Hargrove MS, Pohl NL, Boyd ES, Geesey GG, Hartsel SC, Shafe PH, McEllistrem MT, Kisting CJ, Campbell D, Rao V, De La Mora AM, DiSpirito AA. 2006. Spectral, kinetic, and thermodynamic properties of Cu(I) and Cu(II) binding by methanobactin from *Methylosinus trichosporium* OB3b. Biochemistry 45:1442–1453. https://doi.org/10.1021/bi051815t.
61. Bandow N, Gilles VS, Freesmeier B, Semrau JD, Krentz B, Gallaghe W, McEllistrem MT, Hartse SC, Cho DW, Hargrove MS, Heard TM, Chesner LM, Braunreiter KM, Cao BV, Gavitt MM, Hoopes JZ, Johnson JM, Polster EM, Schoenick BD, Umlauf AM, DiSpirito AA. 2012. Spectral and copper binding properties of methanobactin from the facultative methanotroph *Methylocystis* strain SB2. J Inorg Biochem 110:72–82. https://doi.org/10.1016/j.jinorgbio.2012.02.002.
62. El Ghazouani A, Baslé A, Firbank SJ, Knapp CW, Gray J, Graham DW, Dennison C. 2011. Copper-binding properties and structures of methanobactins from *Methylosinus trichosporium* OB3b. Inorg Chem 50:1378–1391. https://doi.org/10.1021/ic101965j.
63. Pesch M-L, Christl I, Hoffmann M, Kraemer SM, Kretzschmar R. 2012. Copper complexation of methanobactin isolated from *Methylosinus trichosporium*: OB3b: pH-dependent speciation and modeling. J Inorg Biochem 116:55–62. https://doi.org/10.1016/j.jinorgbio.2012.07.008.
64. DiSpirito AA, Semrau JD, Murrell JC, Gallagher WH, Dennison C, Vuilleumier S. 2016. Methanobactin and the link between copper and bacterial methane oxidation. Microbiol Mol Biol Rev 80:387–409. https://doi.org/10.1128/MMBR.00058-15.
65. Krentz BD, Mulheron HJ, Semrau JD, DiSpirito AA, Bandow NL, Haft DH, Vuilleumier S, Murrell JC, McEllistrem MT, Hartsel SC, Gallagher WH. 2010. A comparison of methanobactins from *Methylosinus trichosporium* OB3b and *Methylocystis* strain SB2 predicts methanobactins are synthesized from diverse peptide precursors modified to create a common core for binding and reducing copper ions. Biochemistry 49:10117–10130. https://doi.org/10.1021/bi1014375.
66. Kenney GE, Goering AW, Ross MO, DeHart CJ, Thomas PM, Hoffman BM, Kelleher NL, Rosenzweig AC. 2016. Characterization of methanobactin from *Methylosinus* sp. SW4. J Am Chem Soc 138:11124–11127. https://doi.org/10.1021/jacs.6b06821.
67. Kim HJ, Galeva N, Larive CK, Alterman M, Graham DW. 2005. Purification and physical-chemical properties of methanobactin: a chalkophore from *Methylosinus trichosporium* OB3b. Biochemistry 44:5140–5148. https://doi.org/10.1021/bi047367r.
68. Gu W, Farhan U-HM, Baral BS, Turpin EA, Bandow NL, DiSpirito AA, Lichtmannegger J, Kremmer E, Zischka H, Semaru JD. 2016. A TonB dependent transporter is responsible for methanobactin uptake by *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 82:1917–1923. https://doi.org/10.1128/AEM.03884-15.
69. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE. 2015. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10:845–858. https://doi.org/10.1038/nprot.2015.053.
70. Hegyi H, Gerstein M. 1999. The relationship between protein structure and function: a comprehensive survey with application to the yeast genome. J Mol Biol 288:147–164. https://doi.org/10.1006/jmbi.1999.2661.
71. Wierenga RK. 2001. The TIM-barrel fold: a versatile framework for efficient enzymes. FEBS Lett 492:193–198. https://doi.org/10.1016/S0014-5793(01)02236-0.
72. Kilstrup M, Hanmer K, Jensen RJ, Martinussen JJ. 2005. Nucleotide metabolism and its control in lactic acid bacteria. FEMS Microbiol Rev 29:555–590. https://doi.org/10.1016/j.fmrre.2005.04.006.
73. Kenney GE, Rosenzweig AC. 2013. Genome mining for methanobactin. BMC Biol 11:17. https://doi.org/10.1186/1741-7007-11-17.
74. Semrau JD, Jagadevan S, DiSpirito AA, Khalifa A, Scanlan J, Bergman BH, Freemeier BC, Baral BS, Bandow NL, Vorobev A, Haft DH, Vuilleumier S, Murrell JC. 2013. Methanobactin and MmoD work in concert to act as the 'copper switch' in methanotrophs. Environ Microbiol 15:3077–3086. https://doi.org/10.1111/1462-2920.12150.
75. Gu W, Baral BS, DiSpirito AA, Semrau JD. 2017. An aminotransferase is responsible for the deamination of the *N*-terminal leucine and required for formation of oxazolone ring A in methanobactin of *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 83:e02619–16. https://doi.org/10.1128/AEM.02619-16.
76. McIntire WS, Wemmer DE, Chistoserdov A, Lidstrom ME. 1991. A new cofactor in a prokaryotic enzyme: tryptophan tryptophylquinone as the redox prosthetic group of methylamine dehydrogenase. Science 252:817–824. https://doi.org/10.1126/science.2028257.
77. Bishop GR, Brooks HB, Davidson VL. 1996. Evidence for a tryptophan tryptophylquione aminosemiquione intermediate in the physiologic reaction between methylamine dehydrogenase and amicyanin. Biochemistry 35:8948–8954. https://doi.org/10.1021/bi960404x.
78. Davidson VL. 2001. Pyrroloquinoline quinone (PQQ) from methanol dehydrogenase and tryptophan tryptophyloquinone (TTQ) from methylamine dehydrogenase. Adv Prot Chem 58:95–140. https://doi.org/10.1016/S0065-3233(01)58003-1.
79. Davidson VL. 2007. Protein-derived cofactors. Expanding the scope of post-translational modifications. Biochemistry 48:2583–2592.
80. Wilmot CM, Yukl ET. 2013. MauG: a di-heme enzyme requied for methylamine dehydrogenase maturation. Dalton Trans 42:1327–1335. https://doi.org/10.1039/C2DT32059B.
81. Haft DH, Selengut JD, Richter RA, Harkins D, Basu MK, Beck E. 2013. TIGRFAMs and genome properties in 2013. Nucleic Acids Res 41:D387–D395. https://doi.org/10.1093/nar/gks1234.
82. Borodina E, Nichol T, Dumont MG, Smith TJ, Murrell JC. 2007. Mutagenesis of the "leucine gate" to explore the basis of catalytic versatility in soluble methane monooxygenase. Appl Environ Microbiol 73:6460–6467. https://doi.org/10.1128/AEM.00823-07.
83. Yan X, Chu F, Puri AW, Fu Y, Lidstrom ME. 2016. Electroporation-based genetic manipulation in type I methanotrophs. Appl Environ Microbiol 82:2062–2069. https://doi.org/10.1128/AEM.03724-15.
84. Gu W, Semrau JD. 2017. Copper and cerium-regulated gene expression in *Methylosinus trichosporium* OB3b. Appl Microbiol Biotechnol 101:8499–8516. https://doi.org/10.1007/s00253-017-8572-2.
85. Vita N, Landolfi G, Baslé A, Platsaki S, Lee J, Waldron KJ, Dennison C. 2016. Bacterial cytosolic proteins with a high capacity for Cu(I) that protect against copper toxicity. Sci Rep 6:39065. https://doi.org/10.1038/srep39065.
86. Vita N, Platsaki S, Basle A, Allen SJ, Paterson NG, Crombie AT, Murrell JC, Waldron KJ, Dennison C. 2015. A four-helix bundle stores copper for methane oxidation. Nature 525:140–143. https://doi.org/10.1038/nature14854.
87. Munson GP, Lam DL, Outten FW, O'Halloran TV. 2000. Identification of a copper-responsive two-component system on the chromosome of *Escherichia coli* K-12. J Bacteriol 182:5864–5871. https://doi.org/10.1128/JB.182.20.5864-5871.2000.
88. Karlsen OA, Lillehaug JR, Jensen HB. 2008. The presence of multiple *c*-type cytochromes at the surface of the methanotrophic bacterium *Methylococcus capsulatus* (Bath) is regulated by copper. J Biol Chem 279:51544.
89. Larsen Ø, Karlsen OA. 2016. Transcriptomic profiling of *Methylococcus capsulatus* (Bath) during growth with two different methane monooxygenases. Microbiologyopen 5:254–267. https://doi.org/10.1002/mbo3.324.
90. Pham AN, Xing G, Miller CJ, Waite TD. 2013. Fenton-like copper redox chemistry revisited: hydrogen peroxide and superoxide mediation of copper-catalyzed oxidant production. J Catal 301:54–64. https://doi.org/10.1016/j.jcat.2013.01.025.
91. Macomber L, Imlay JA. 2009. The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proc Natl Acad Sci U S A 106:8344–8349. https://doi.org/10.1073/pnas.0812808106.
92. Chang J, Gu W, Park D, Semrau JD, DiSpirito AA, Yoon S. 12 January 2018. Methanobactin from *Methylosinus trichosporium* OB3b inhibits $N_2O$ reduction in denitrifiers. ISME J https://doi.org/10.1038/s41396-017-0022-8.
93. Scott RA, Zumft WG, Coyle CL, Dooley DM. 1989. *Pseudomonas stutzeri* $N_2O$ reductase contains $Cu_A$-type sites. Proc Natl Acad Sci U S A 86:4082–4086. https://doi.org/10.1073/pnas.86.11.4082.
94. Amaral JA, Knowles R. 1995. Growth of methanotrophs in methane and oxygen counter gradients. FEMS Microbiol Lett 126:215–220. https://doi.org/10.1111/j.1574-6968.1995.tb07421.x.
95. Reim A, Luke C, Krause S, Pratscher J, Frenzel P. 2012. One millimetre makes the difference: high-resolution analysis of methane-oxidizing bacteria and their specific activity at the oxic-anoxic interface in a

flooded paddy soil. ISME J 6:2128–2139. https://doi.org/10.1038/ismej.2012.57.
96. Maeda M, Zhao B, Ozaki Y, Yoneyama T. 2003. Nitrate leaching in an Andisol treated with different types of fertilizers. Environ Pollut 121:477–487. https://doi.org/10.1016/S0269-7491(02)00233-6.
97. Zumft W. 1997. Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61:533–616.
98. Vorobev A, Jagadevan S, Baral BS, DiSpirito AA, Freemeier BC, Bergman BH, Bandow NL, Semrau JD. 2013. Detoxification of mercury by methanobactin from *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 79:5918–5926. https://doi.org/10.1128/AEM.01673-13.
99. Baral BS, Bandow NL, Vorobev A, Freemeier BC, Bergman BH, Herdendorf T, Fuentes N, Ellias L, Turpin E, Semrau JD, DiSpirito AA. 2014. Mercury binding by methanobactin from *Methylocystis* strain SB2. J Inorg Biochem 141:161–169. https://doi.org/10.1016/j.jinorgbio.2014.09.004.
100. Lu X, Gu W, Zhao L, Fagan UHM, DiSpirito AA, Semrau JD, Gu B. 2017. Methylmercury uptake and degradation by methanotrophs. Sci Adv 3:e1700041. https://doi.org/10.1126/sciadv.1700041.
101. Begley TP, Walts AE, Walsh CT. 1986. Mechanistic studies of a protonolytic organomercurial cleaving enzyme: bacterial organomercurial lyase. Biochemistry 25:7192–7200. https://doi.org/10.1021/bi00370a064.
102. Ve T, Mathisen K, Helland R, Karlsen OA, Fjellbirkeland A, Røhr Å K, Andersson KK, Pedersen RB, Lillehaug JR, Jensen HB. 2012. The *Methylococcus capsulatus* (Bath) secreted protein, MopE*, binds both reduced and oxidized copper. PLoS One 7:e43146. https://doi.org/10.1371/journal.pone.0043146.
103. Helland R, Fjellbirkeland A, Karlsen OA, Ve T, Lillehaug JR, Jensen HB. 2008. An oxidized tryptophan facilitates copper binding in Methylococcus capsulatus-secreted protein MopE. J Biol Chem 283:13897–13904. https://doi.org/10.1074/jbc.M800340200.
104. Karlsen OA, Berven FS, Stafford GP, Larsen Ø, Murrell JC, Jensen HB, Fjellbirkeland A. 2003. The surface-associated and secreted MopE protein of *Methylococcus capsulatus* (Bath) responds to changes in the concentration of copper in the growth medium. Appl Environ Microbiol 69:2386–2388. https://doi.org/10.1128/AEM.69.4.2386-2388.2003.
105. Fjellbirkeland A, Kleivdal H, Joergensen C, Thestrup H, Jensen HB. 1997. Outer membrane proteins of *Methylococcus capsulatus* (Bath). Arch Microbiol 168:128–135. https://doi.org/10.1007/s002030050478.
106. Tyler G. 2005. Rare earth elements in soil and plant systems–a review. Plant Soil 267:191–206. https://doi.org/10.1007/s11104-005-4888-2.
107. Gupta CK, Krishnamurthy N. 2016. Extractive metallurgy of rare earths. CRC Press, Boca Raton, FL.
108. Johnson GT, Kyker GC. 1961. Fission-product and cerium uptake by bacteria, yeasts, and molds. J Bacteriol 81:733–740.
109. Lim S, Franklin SJ. 2004. Lanthanide-binding peptides and the enzymes that might have been. Cell Mol Life Sci 61:2184–2188. https://doi.org/10.1007/s00018-004-4156-2.
110. Anthony C. 2004. The quinoprotein dehydrogenases for methanol and glucose. Arch Biochem Biophys 428:2–9. https://doi.org/10.1016/j.abb.2004.03.038.
111. Anthony C, Williams P. 2003. The structure and mechanism of methanol dehydrogenase. Biochim Biophys Acta 1647:18–23. https://doi.org/10.1016/S1570-9639(03)00042-6.
112. Anthony C, Ghosh M. 1998. The structure and function of the PQQ-containing quinoprotein dehydrogenases. Prog Biophys Mol Biol 69:1–21. https://doi.org/10.1016/S0079-6107(97)00020-5.
113. Anthony C. 1992. The structure of bacterial quinoprotein dehydrogenases. Int J Biochem 24:29–30. https://doi.org/10.1016/0020-711X(92)90226-Q.
114. Ras J, Reijnders WNM, Van Spanning RJM, Harms N, Oltmann LF, Stouthamer AH. 1991. Isolation, sequencing, and mutagenesis of the gene encoding cytochrome $c_{553i}$ of *Paracoccus denitrificans* and characterization of the mutant strain. J Bacteriol 173:6971–6979. https://doi.org/10.1128/jb.173.21.6971-6979.1991.
115. Chistoserdova L, Lidstrom ME. 1997. Molecular and mutational analysis of a DNA region separating two methylotrophy gene clusters in *Methylobacterium extorquens* AM1. Microbiology 143:1729–1736. https://doi.org/10.1099/00221287-143-5-1729.
116. Harms N, Ras J, Reijnders WNM, Stouthamer AH, Van Spanning RJM. 1996. Genetics of $C_1$ metabolism regulation in *Paracoccus denitrificans*, p 126–132. *In* Lidstrom ME, Tabita FR (ed), Microbial growth on $C_1$ compounds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
117. Wilson SM, Gleisten MP, Donohue TJ. 2008. Identification of proteins involved in formaldehyde metabolism by *Rhodobacter sphaeroides*. Microbiology 154:296–305. https://doi.org/10.1099/mic.0.2007/011346-0.
118. Schmidt S, Christen P, Kiefer P, Vorholt JA. 2010. Functional investigation of methanol dehydrogenase-like protein XoxF in *Methylobacterium extorquens* AM1. Microbiology 156:2575–2586. https://doi.org/10.1099/mic.0.038570-0.
119. Bosch G, Skovran E, Xia Q, Wang T, Taub F, Miller JA, Lidstrom ME, Hackett M. 2008. Comprehensive proteomics of *Methylobacterium extorquens* AM1 metabolism under single carbon and nonmethylotrophic conditions. Proteomics 8:3494–3505. https://doi.org/10.1002/pmic.200800152.
120. Hibi Y, Asai K, Arafuka H, Hamajima M, Iwama T, Kawai K. 2011. Molecular structure of $La^{3+}$-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. J Biosci Bioeng 111:547–549. https://doi.org/10.1016/j.jbiosc.2010.12.017.
121. Fitriyanto NA, Fushimi M, Matsunaga M, Pertiwiningrum A, Iwama T, Kawai K. 2011. Molecular structure and gene analysis of $Ce^{3+}$-induced methanol dehydrogenase of *Bradyrhizobium* sp. MAFF211645. J Biosci Bioeng 111:613–617. https://doi.org/10.1016/j.jbiosc.2011.01.015.
122. Nakagawa T, Mitsui R, Tani A, Sasa K, Tashiro S, Iwama T, Hayakawa T, Kawai K. 2012. A catalytic role of XoxF1 as $La^{3+}$-dependent methanol dehydrogenase in *Methylobacterium extorquens* strain AM1. PLoS One 7:e50480. https://doi.org/10.1371/journal.pone.0050480.
123. Pol A, Barends TRM, Dietl A, Khadem AF, Eygensteyn J, Jetten MMS, Op den Camp HJ. 2014. Rare earth metals are essential for methanotrophic life in volcanic mudpots. Environ Microbiol 16:255–264. https://doi.org/10.1111/1462-2920.12249.
124. Farhan Ul Haque M, Gu W, DiSpirito AA, Semrau JD. 2016. Marker exchange mutagenesis of *mxaF*, encoding the large subunit of the Mxa methanol dehydrogenase, in *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 82:1549–1555. https://doi.org/10.1128/AEM.03615-15.
125. Farhan Ul Haque M, Kalidass B, Bandow NL, Turpin E, DiSpirito AA, Semrau JD. 2015. Cerium regulates expression of alternative methanol dehydrogenases in *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 81:7446–7554. https://doi.org/10.1128/AEM.02542-15.
126. Vu HN, Subuyi GA, Viiayakumar S, Good NM, Martinez-Gomez NC, Skovran E. 2016. Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. J Bacteriol 198:1250–1259. https://doi.org/10.1128/JB.00937-15.
127. Gu W, Farhan Ul Haque M, DiSpirito AA, Semrau JD. 2016. Uptake and effect of rare earth elements on gene expression in *Methylosinus trichosporium* OB3b. FEMS Microbiol Lett 363:1–6. https://doi.org/10.1093/femsle/fnw129.
128. Chu F, Lidstrom ME. 2016. XoxF acts as the predominant methanol dehydrogenase in the type I methanotroph *Methylomicrobium buryatense*. J Bacteriol 198:1317–1325. https://doi.org/10.1128/JB.00959-15.
129. Dahle JT, Arai Y. 2015. Environmental geochemistry of cerium: applications and toxicology of cerium oxide nanoparticles. Intl J Environ Res Public Health 12:1253–1278. https://doi.org/10.3390/ijerph120201253.
130. Bultreys A, Gheysen I, Maraite H, de Hoffmann E. 2001. Characterization of fluorescent and nonfluorescent peptide siderophores produced by *Pseudomonas syringae* strains and their potential use in strain identification. Appl Environ Microbiol 67:1718–1727. https://doi.org/10.1128/AEM.67.4.1718-1727.2001.
131. Drever JI, Stillings LL. 1997. The role of organic acids in mineral weathering. Colloids Surf A 120:167–181. https://doi.org/10.1016/S0927-7757(96)03720-X.
132. Duckworth OW, Sposito G. 2005. Siderophore-manganese(III) interactions II. Manganite dissolution promoted by desferrioxamine. B Environ Sci Technol 39:6045–6051.
133. Ehrlich HL. 1997. Microbes and metals. Appl Microbiol Biotechnol 48:687–692. https://doi.org/10.1007/s002530051116.
134. Lundgren DG, Silver M. 1980. Ore leaching by bacteria. Annu Rev Microbiol 34:263–283. https://doi.org/10.1146/annurev.mi.34.100180.001403.
135. Haack EA, Johnston CT, Maurice PA. 2008. Mechanisms of siderophore sorption to smectite and siderophore-enhanced release of structural $Fe^{3+}$. Geochim Cosmochim Acta 72:3381–3397. https://doi.org/10.1016/j.gca.2008.03.027.
136. Neilands JB. 1995. Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726. https://doi.org/10.1074/jbc.270.45.26723.
137. White C, Sayer JA, Gadd GM. 1997. Microbial solubilization and immo-

bilization of toxic metals: key biogeochemical processes for treatment of contamination. FEMS Microbiol Rev 20:503–516. https://doi.org/10.1111/j.1574-6976.1997.tb00333.x.
138. Beard SJ, Hashim R, Wu G, Binet MRB, Hughes MN, Poole RK. 2000. Evidence for the transport of zinc(II) ions via the Pit inorganic phosphate transport system in *Escherichia coli*. FEMS Microbiol Lett 184:231–235. https://doi.org/10.1111/j.1574-6968.2000.tb09019.x.
139. Jackson RJ, Binet MRB, Lee LJ, Ma R, Graham AI, McLeod CW, Poole RK. 2008. Expression of the PitA phosphate/metal transporter of *Escherichia coli* is responsive to zinc and inorganic phosphate levels. FEMS Microbiol Lett 289:219–224. https://doi.org/10.1111/j.1574-6968.2008.01386.x.
140. Kirsten A, Herzberg M, Voigt A, Seravalli J, Grass G, Scherer J, Nies DH. 2011. Contributions of five secondary metal uptake systems to metal homeostasis of *Cupriavidus metallidurans* CH34. J Bacteriol 193:4652–4663. https://doi.org/10.1128/JB.05293-11.
141. Yoon S, Kraemer SM, DiSpirito AA, Semrau JD. 2010. An assay for screening microbial cultures for chalkophore production. Environ Microbiol Rep 2:295–303. https://doi.org/10.1111/j.1758-2229.2009.00125.x.
142. Whittenbury R, Phillips KC, Wilkinson JF. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 61:205–218. https://doi.org/10.1099/00221287-61-2-205.
143. Kalyuzhnaya MG, Yang S, Rozova ON, Smalley NE, Clubb J, Lamb A, Gowda GAN, Raftery D, Fu Y, Bringel F, Vuilleumier S, Beck DAC, Trotsenko YA, Khmelenina VN, Lidstrom ME. 2013. Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. Nat Commun 4:2785. https://doi.org/10.1038/ncomms3785.
144. Martinez-Gomez NC, Vu HN, Skovran E. 2016. Lanthanide chemistry: from coordination in chemical complexes shaping our technology to coordination in enzymes shaping bacterial metabolism. Inorg Chem 55:10083–10089. https://doi.org/10.1021/acs.inorgchem.6b00919.
145. Chu F, Beck DAC, Lidstrom ME. 2016. MxaY regulates the lanthanide-mediated methanol dehydrogenase switch in *Methylomicrobium buryatense*. PeerJ 2016:e2435. https://doi.org/10.7717/peerj.2435.
146. Keltjens JT, Pol A, Reimann J, Op den Camp HJM. 2014. PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. Appl Microbiol Biotechnol 98:6163–6183. https://doi.org/10.1007/s00253-014-5766-8.
147. Taubert M, Grob C, Howat AM, Burns OJ, Dixon JL, Chen Y, Murrell JC. 2015. XoxF encoding an alternative methanol dehydrogenase is widespread in coastal marine environments. Environ Microbiol 17:3937–3948. https://doi.org/10.1111/1462-2920.12896.
148. Grob C, Taubert M, Howat AM, Burns OJ, Dixon JL, Richnow HH, Jehmlich N, von Bergen M, Chen Y, Murrell JC. 2015. Combining metagenomics with metaproteomics and stable isotope probing reveals metabolic pathways used by a naturally occurring marine methylotroph. Environ Microbiol 17:4007–4018. https://doi.org/10.1111/1462-2920.12935.
149. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Von Mering C, Vorholt JA. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc Natl Acad Sci U S A 106:16428–16433. https://doi.org/10.1073/pnas.0905240106.
150. del Rocío Bustillos-Cristales M, Corona-Gutierrez I, Castañeda-Lucio M, Águila-Zempoaltécatl C, Seynos-García E, Hernández-Lucas I, Muñoz-Rojas J, Medina-Aparicio L, Fuentes-Ramírez LE. 2017. Culturable facultative methylotrophic bacteria from the cactus *Neobuxbaumia macrocephala* possess the locus *xoxF* and consume methanol in the presence of $Ce^{3+}$ and $Ca^{2+}$. Microbes Environ 32:244–251. https://doi.org/10.1264/jsme2.ME17070.
151. Ramachandran A, Walsh DA. 2015. Investigation of XoxF methanol dehydrogenases reveals new methylotrophic bacteria in pelagic marine and freshwater ecosystems. FEMS Microbiol Ecol 91:fiv105. https://doi.org/10.1093/femsec/fiv105.
152. Sowell SM, Abraham PE, Shah M, Verberkmoes NC, Smith DP, Barofsky DF, Giovannoni SJ. 2011. Environmental proteomics of microbial plankton in a highly productive coastal upwelling system. ISME J 5:856–865. https://doi.org/10.1038/ismej.2010.168.
153. Mattes TE, Nunn BL, Marshall KT, Proskurowski G, Kelley DS, Kawka OE, Goodlett DR, Hansell DA, Morris RM. 2013. Sulfur oxidizers dominate carbon fixation at a biogeochemical hot spot in the dark ocean. ISME J 7:2349–2360. https://doi.org/10.1038/ismej.2013.113.
154. Vekeman B, Speth D, Wille J, Cremers G, De Vos P, Op den Camp HJM, Heylen K. 2016. Genome characteristics of two novel type I methanotrophs enriched from North Sea sediments containing exclusively a lanthanide-dependent $XoxF_5$-type methanol dehydrogenase. Microb Ecol 72:503–509. https://doi.org/10.1007/s00248-016-0808-7.
155. Giovannoni SJ, Hayakawa DH, Tripp HJ, Stingl U, Givan SA, Cho JC, Oh HM, Kitner JB, Vergin KL, Rappé MS. 2008. The small genome of an abundant coastal ocean methylotroph. Environ Microbiol 10:1771–1782. https://doi.org/10.1111/j.1462-2920.2008.01598.x.
156. Chistoserdova L. 2011. Modularity of methylotrophy, revisited. Environ Microbiol 13:2603–2622. https://doi.org/10.1111/j.1462-2920.2011.02464.x.
157. Hou S, Makarova KS, Saw JHW, Senin P, Ly BV, Zhou Z, Ren Y, Wang J, Galperin MY, Omelchenko MV, Wolf YI, Yutin N, Koonin EV, Stott MB, Mountain BW, Crowe MA, Smirnova AV, Dunfield PF, Feng L, Wang L, Alam M. 2008. Complete genome sequence of the extremely acidophilic methanotroph isolate V4, *Methylacidiphilum infernorum*, a representative of the bacterial phylum *Verrucomicrobia*. Biol Direct 3:26. https://doi.org/10.1186/1745-6150-3-26.
158. Kalyuzhnaya MG, Hristova KR, Lidstrom ME, Chistoserdova L. 2008. Characterization of a novel methanol dehydrogenase in representatives of *Burkholderiales*: implications for environmental detection of methylotrophy and evidence for convergent evolution. J Bacteriol 190:3817–3823. https://doi.org/10.1128/JB.00180-08.
159. Gifford SM, Becker JW, Sosa OA, Repeta DJ, DeLong EF. 2016. Quantitative transcriptomics reveals the growth- and nutrient-dependent response of a streamlined marine methylotroph to methanol and naturally occurring dissolved organic matter. mBio 7:e01279-16. https://doi.org/10.1128/mBio.01279-16.
160. Shiller AM, Chan EW, Joung DJ, Redmond MC, Kessler JD. 2017. Light rare earth element depletion during Deepwater Horizon blowout methanotrophy. Sci Rep 7:10389. https://doi.org/10.1038/s41598-017-11060-z.
161. Krause SMB, Johnson T, Karunaratne YS, Fu Y, Beck DAC, Chistoserdova L, Lidstrom ME. 2017. Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. Proc Natl Acad Sci U S A 114:358–363. https://doi.org/10.1073/pnas.1619871114.
162. Ho A, de Roy K, Thas O, De Neve J, Hoefman S, Vandamme P, Heylen K, Boon N. 2014. The more, the merrier: heterotroph richness stimulates methanotrophic activity. ISME J 8:1945–1948. https://doi.org/10.1038/ismej.2014.74.
163. Iguchi H, Yurimoto H, Sakai Y. 2011. Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. Appl Environ Microbiol 77:8509–8515. https://doi.org/10.1128/AEM.05834-11.
164. Pruesse E, Peplies J, Glöckner FO. 2012. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. https://doi.org/10.1093/bioinformatics/bts252.
165. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054.
166. Behling LA, Hartsel SC, Lewis DE, DiSpirito AA, Choi DW, Masterson LR, Veglia G, Gallagher WH. 2008. NMR, mass spectrometry and chemical evidence reveal a different chemical structure for methanobactin that contains oxazolone rings. J Am Chem Soc 130:12604–12605. https://doi.org/10.1021/ja804747d.
167. Bandow NL, Gallagher WH, Behling L, Choi DW, Semrau JD, Hartsel SC, Gilles VS, DiSpirito AA. 2011. Isolation of methanobactin from the spent media of methane-oxidizing bacteria. Methods Enzymol 495:259–269. https://doi.org/10.1016/B978-0-12-386905-0.00017-6.