CHAPTER TWO

# Microbial mercury transformations: Molecules, functions and organisms

**Ri-Qing Yu[a,*] and Tamar Barkay[b]**
[a]Department of Biology, University of Texas at Tyler, Tyler, TX, United States
[b]Department of Biochemistry and Microbiology, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States
*Corresponding author: e-mail address: ryu@uttyler.edu

## Contents



## Abstract

Mercury (Hg) methylation, methylmercury (MeHg) demethylation, and inorganic redox transformations of Hg are microbe-mediating processes that determine the fate and cycling of Hg and MeHg in many environments, and by doing so influence the health of humans and wild life. The discovery of the Hg methylation genes, *hgcAB*, in the last decade together with advances in high throughput and genome sequencing methods, have resulted in an expanded appreciation of the diversity of Hg methylating microbes. This review aims to describe experimentally confirmed and recently discovered *hgcAB* gene-carrying Hg methylating microbes; phylogenetic and taxonomic analyses are presented. In addition, the current knowledge on transformation mechanisms, the organisms that carry them out, and the impact of environmental parameters on Hg

*Advances in Applied Microbiology*, Volume 118
ISSN 0065-2164
https://doi.org/10.1016/bs.aambs.2022.03.001

methylation, MeHg demethylation, and inorganic Hg reduction and oxidation is summarized. This knowledge provides a foundation for future action toward mitigating the impact of environmental Hg pollution.

## 1. Introduction

Mercury (Hg) is a highly toxic heavy metal that poses a serious risk to human and environmental health (Guzzi, Ronchi, & Pigatto, 2021; Sall, Diaw, Gningue-Sall, Aaron, & Aaron, 2020; Yang et al., 2020). Mercury is mainly emitted in its inorganic form from geological and anthropogenic sources, such as coal combustion, to the atmosphere where it is subject to global mobilization and deposition in remote areas (Pacyna, 2020). Once inorganic Hg enters rivers, lakes, estuaries, and wetlands, it can be converted to its most toxic form, the neurotoxic methylmercury (MeHg) by microbes and becomes available to the food chains. This in turn leads to bioaccumulation and biomagnification at the top levels of the food chains. When consumed, MeHg could cause detrimental effects to aquatic biota, piscivorous wildlife (Chételat, Ackerman, Eagles-Smith, & Hebert, 2020), and humans (Eagles-Smith et al., 2018; Ha et al., 2017), especially impacting human fetal development (Aaseth, Wallace, Vejrup, & Alexander, 2020). This dynamic of toxicity, emission of inorganic Hg and in situ conversion to MeHg, brings the environmental Hg biogeochemical cycle to the fore (Fig. 1). Any transformation and transport process that takes place in Hg-exposed environments may modulate how much of the deposited Hg is methylated and becomes available to the local food chain. Therefore, these processes are the crucial links between atmospheric Hg emissions and bioavailability of Hg as MeHg in ecosystems and they are the focus of this paper. Here, we highlight the role of microorganisms and their activities in the Hg geochemical cycle (Fig. 1) where they directly and indirectly affect MeHg formation and its degradation.

Effective management to reduce atmospheric Hg emissions and their impact on human and environmental health could be implemented only based on knowledge and understanding of the Hg biogeochemical cycle as culminated in microbial MeHg production. The exploration of Hg biotransformation mechanisms, therefore, will help facilitate remediation strategies to control MeHg production in the environment. Microorganisms affect MeHg production directly by methylating inorganic Hg to MeHg (Jensen & Jernelov, 1969; Yu, Reinfelder, Hines, & Barkay, 2018) and

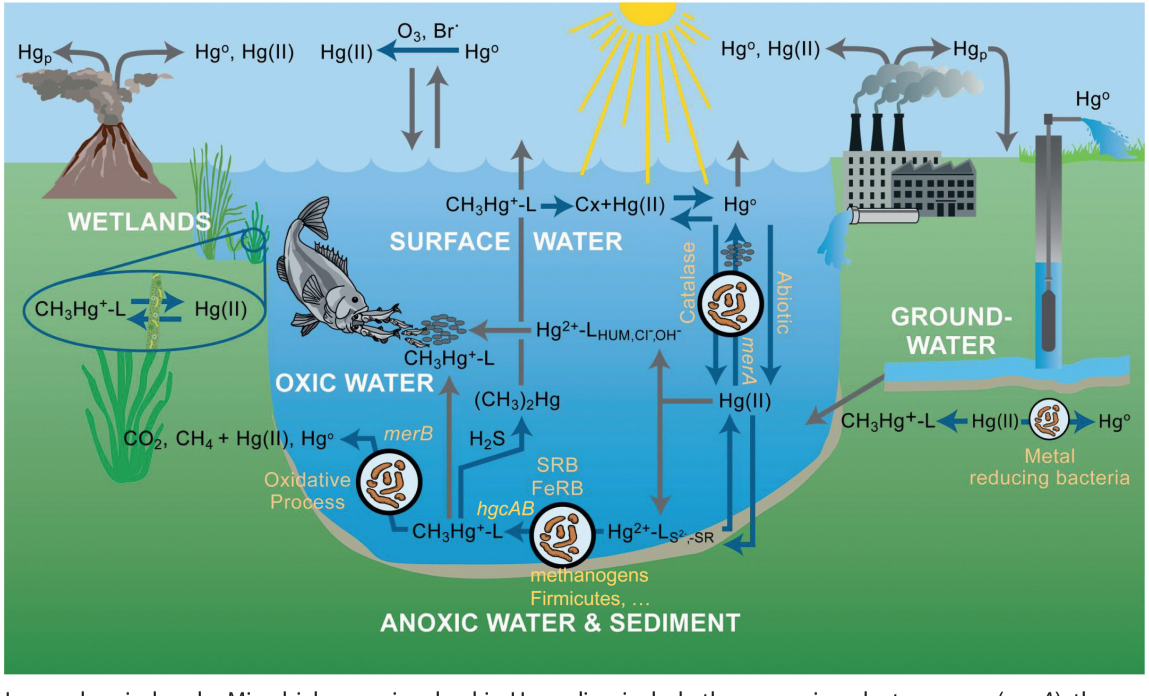


**Fig. 1** The Hg geochemical cycle. Microbial genes involved in Hg cycling include the mercuric reductase gene (*merA*), the organomercury lyase gene (*merB*), and the Hg methylation genes (*hgcAB*). Hg methylating microbes shown consist of sulfate reducing bacteria (SRB) and iron reducing bacteria (FeRB). In addition, methanogens, fermenters and other microbes may also methylate Hg(II). *This figure is revised from Lin, C.-C., Yee, N., Barkay, T., 2012. Microbial transformations in the mercury cycle., in: Liu, G., Cai, Y., O'Driscoll, N. (Eds.), Environmental chemistry and toxicology of mercury. John Wiley & Sons, Inc., pp. 155–191.*

by degrading MeHg (Hines et al., 2000; Spangler, Spigarelli, Rose, & Miller, 1973). While current paradigms attribute Hg methylation largely to anaerobic microbes that possess unique methylation genes (Ma, Du, & Wang, 2019; Parks et al., 2013; Regnell & Watras, 2019) with little contribution from chemical methylation (Weber, 1993), numerous demethylation processes, both biotic and abiotic, have been documented (Barkay & Gu, 2022; Du, Ma, Igarashi, & Wang, 2019). Redox transformations of inorganic Hg indirectly affect the availability of the Hg substrate for methylation (Grégoire & Poulain, 2018; Obrist et al., 2018). Processes that enhance reduction of Hg(II) to volatile Hg(0) may limit methylation (Saouter, Gillman, & Barkay, 1995) while the oxidation of Hg(0) to Hg(II) may enhance methylation (Colombo, Ha, Reinfelder, Barkay, & Yee, 2013; Hu et al., 2013). This intricate system is further complicated by numerous interactions with the physical chemical environment whereby pH, light, salinity and presence of ligands, nutrients, oxidants, and reductants affect Hg bioavailability as well as the activities of methylating microbes (Hsu-Kim, Kucharzyk, Zhang, & Deshusses, 2013; Tang et al., 2020).

Gaps in knowledge on the mechanisms of microbial Hg methylation, MeHg degradation and redox transformations, are substantial. In this review, we particularly address the identity of microbes that partake in these processes, transformation mechanisms, and the genes that encode them. Our main purpose is to explore microbes and genes involved in methylation, MeHg degradation, and inorganic Hg redox transformations. Specifically, we focus on methylating microbes as the discovery of *hgcA* and *hgcB*, the methylation genes (Parks et al., 2013), has resulted in the identification of many new methylating taxa and microbial guilds (Gilmour, Bullock, McBurney, Podar, & Elias, 2018; Gilmour et al., 2013; Gionfriddo, Podar, Gilmour, Pierce, & Elias, 2019). Our final goal is to provide new information relevant to Hg cycling, environmental factors controlling microbial Hg uptake, and mechanisms of microbial Hg transformations, and management interventions.

## 2. Mercury methylation

### 2.1 Hg methylation mechanisms

From a historical perspective, studies on microbial Hg methylation mechanisms could be classified into three periods: (1) An early exploration period of microbial Hg methylation; (2) A preliminary determination of a biochemical pathway; and (3) New discoveries driven by the identification

of the Hg methylation genes, *hgcAB*. Initially, shortly after the Hg methylation phenomenon was discovered in the mid to late 1960s by Jensen and Jernelov (1969), methanogens were hypothesized to be the main methylators. This proposition was based on the fact that the only methyl transfer compound known to donate a negatively charged methyl group, as would be needed for the methylation of the positively charged Hg(II), was methylcorrinoids (methyl $B_{12}$); methyl B12 is common among methanogens where it is essential for methane production (Boone, 1987; Bryant, Wolin, Wolin, & Wolfe, 1967; Wood, Kennedy, & Rosen, 1968). Indeed, cell-free extracts of a methanogen produced MeHg (Wood et al., 1968). However, this hypothesis was rejected later by experiments with saltmarsh sediment incubations in which methylation was inhibited by the sulfate reduction inhibitor, molybdate, confirming sulfate reducing bacteria (SRB) as the principal methylators (Compeau & Bartha, 1985). Later yet, isolates of iron reducing bacteria (FeRB) were identified as another group of major Hg methylators (Fleming, Mack, Green, & Nelson, 2006; Kerin et al., 2006). In the 2010s methanogens were finally confirmed as a novel group of MeHg producers in environmental incubations (Hamelin, Amyot, Barkay, Wang, & Planas, 2011) and in pure cultures (Gilmour et al., 2013; Yu, Reinfelder, Hines, & Barkay, 2013). Most recently, fermenting bacteria were identified as another guild of methylators following the discovery of *hgcAB* gene homologs in their genomes (Gilmour et al., 2013).

Studies on the biochemical mechanism of Hg methylation by SRB were initiated in Richard Bartha's lab using *Desulfovibrio desulfuricans* strain LS as a model organism. While this strain, originally isolated from saltmarsh sediment, was later lost, strain *D. desulfuricans* ND132, now renamed *Psudodesulfovibrio mercurii* ND132 (Gilmour et al., 2021), is an excellent replacement. Berman, Chase, and Bartha (1990) reported that when [3-$^{14}$C]-serine was added to the culture medium of strain LS, 95% of the specific activity of the substrate was retained by the $^{14}$C-MeHg product. This study further confirmed that cobalamin played a key role in methyl transfer to Hg(II) by using propyl iodide as an inhibitor of transmethylation. The authors concluded that serine was the methyl donor to the cobalamin. Choi and Bartha (1993) reported that when $^{57}$Co was added to strain LS cultures, Hg methylation was stimulated 2.5-fold and 97% of the $^{57}$Co was associated with cobalamin. By using crude cell extracts of strain LS, Choi, Chase, and Bartha (1994a, 1994b) further showed that methylcobalamin is a key catalyst in the Hg methylation process and proposed that methylation of Hg by *D. desulfuricans* proceeded through the acetyl-CoA synthase pathway. However, using

chloroform as an inhibitor of the acetyl-CoA pathway, Ekstrom, Morel, and Benoit (2003) showed that only complete-oxidizer SRB, those that convert acetate and other fatty acids to $CO_2$ (e.g., *Desulfococcus*, *Desulfosarcina*, and *Desulfobacterium*), and *D. desulfuricans* LS, an incomplete oxidizer (Choi et al., 1994a), used the acetyl-CoA pathway for Hg methylation. Incomplete oxidizers, those unable to convert acetate to $CO_2$ (e.g., *Desulfovibrio*, *Desulfobulbus*) did not. Strain *D. desulfuricans*, an incomplete oxidizer, therefore, was an exception. Indeed, Choi et al. (1994a) detected very low levels of the acetyl-CoA pathway enzymes in cell extracts of strain LS and noted that this pathway could only play a minor metabolic role. Thus, MeHg synthesis by complete oxidizer SRB is mainly catalyzed by a $B_{12}$ (or cobalamin)-containing methyltransferase, while MeHg synthesis by incomplete oxidizers like *D. africanus* (now *Desulfocurvibacter africanus*) (Spring et al., 2019) is mediated by a $B_{12}$-independent methyltransferase (Ekstrom & Morel, 2008).

The Hg methylation genes (*hgcAB*) were discovered almost a decade ago (Parks et al., 2013) and have contributed greatly toward a better understanding of the molecular mechanisms of methylation and the functions of the encoded proteins, HgcAB. Yet, much remains to be deciphered. By examining protein homology (BLAST search), HgcA, encoded by the *hgcA* gene, belongs to a subset of the CO dehydrogenase/acetyl-CoA synthase delta subunit family, generally involved in carbon metabolism especially in methyl transfer reactions (Parks et al., 2013). As a corrinoid protein, HgcA contains a cytosolic corrinoid binding domain (CBD), and a transmembrane domain (TMD). The cytosolic CBD likely partakes in transfer of the methyl group from carbon substrate metabolism to Hg(II), while the TMD is likely responsible for Hg uptake and cellular MeHg efflux (Parks et al., 2013; Poulain & Barkay, 2013; Regnell & Watras, 2019). HgcB, encoded by the *hgcB* gene, is a dicluster ferredoxin (iron-sulfur cluster protein) with three additional conserved cysteine residues at the C terminus, and likely acts as an electron donor to reduce the cobalt ion of HgcA thus refreshing its enzymatic activity, and to bind and deliver Hg(II) to HgcA for methylation (Date et al., 2019; Parks et al., 2013; Regnell & Watras, 2019; Smith et al., 2015). Recent gene overexpression study and modeling by Cooper et al. (2020) indicated little interaction between the two HgcA domains, the CBD and TMD, while HgcB forms extensive contacts with both. Based on these observations, the authors suggested a model whereby the three C-terminus-conserved cysteines in HgcB are involved in transporting Hg(II) to the HgcA-bound coronoid and in releasing MeHg (Cooper et al., 2020).

The evolutionary adaptation mechanism and the physiological advantages of microbial Hg methylation have been a riddle for decades. One of the two hypotheses for the evolutionary benefits is that, microbes methylate Hg by a co-metabolism pathway in which HgcA and HgcB catalyze the methyl transfer to Hg(II) during cellular carbon metabolism, thus, methylating Hg(II) by accident as a side product (Barkay & Wagner-Döbler, 2005; Parks et al., 2013). The second hypothesis is that methylation serves as a detoxification process in which methylation confers Hg resistance to cells. Schaefer et al. (2011) proposed that rapid MeHg export during Hg methylation could avoid buildup and toxicity of Hg that is accidentally taken up by a transport system for essential metals. The physiological function of such metabolic activities is likely to expedite the extracellular excretion of toxic Hg once inorganic Hg(II) is converted to MeHg inside the cell, even though MeHg could be as toxic to microorganisms as Hg(II) (Schaefer et al., 2011). However, based on Hg(II) toxicity comparison between methylating and non-methylating *Desulfovibrio* species, it seems that the ability to produce MeHg does not confer Hg resistance (Gilmour et al., 2011). A recent study by Qian et al. (2018) revealed that the expression levels of *hgcAB* genes were not induced by spiking Hg(II) in cultures of *P. mercurii* ND132$^{T}$, supporting the conclusion that there is no obvious correlation between the Hg resistance and methylation gene expression. While these conclusions are plausible, a comparison of resistance levels between wild type methylators and their *hgcAB* deletion mutants has not been reported.

## 2.2 Geochemical factors influencing Hg methylation

Mercury methylation in the environment is a complex process impacted by a variety of biotic and abiotic factors, and it is generally determined by Hg bioavailability due to the geochemical speciation of inorganic Hg, activities of Hg methylating microbes, and degradation of MeHg (Hsu-Kim et al., 2013; Lin, Yee, & Barkay, 2012; Regnell & Watras, 2019). The microbial cellular uptake of inorganic Hg, a process that is usually governed by chemical speciation, is the initial and crucial step for MeHg production. Therefore, studies on the geochemical speciation of Hg(II) in the environment are crucial to understand Hg bioavailability for methylating microorganisms. In natural waters, Hg(II) usually complexes with chloride, sulfides, or dissolved organic matter, existing as a mixture of dissolved, colloidal, and particulate phases. In sediment or porewater, Hg(II) is likely associated with particles in the form of weakly sorbed, amorphous, or nanostructured

species. Although the matrices and mechanisms of Hg speciation are still largely unknown, sulfides and natural organic matter have become the two predominant factors considered to affect bioavailability of Hg(II) to microbial Hg methylation (Hsu-Kim et al., 2013). In this section, we will focus on several major geochemical factors which likely influence Hg methylation.

### 2.2.1 $SO_4^{2-}$, $S^{2-}$, and $HS^-$

The geochemical speciation of inorganic sulfur in aquatic system and soil phases is usually separated into sulfate, acid-volatile sulfide, elemental S, and pyrite-S, dominated by sulfate ($SO_4^{2-}$) and sulfide ($S^{2-}$, $HS^-$) (Colin et al., 2020; Fossing & Jørgensen, 1989). As the most oxidized form of sulfur, sulfate is the electron acceptor in anaerobic respiration for sulfate reducing bacteria (SRB). Therefore, analyses of sulfate levels in sediment, porewater or wetland soil are crucial in the preliminary exploration of Hg methylation mechanisms and further identification of the major Hg methylating microbes. Amendment of sulfate to sediment or soil in microcosms could enhance microbial Hg methylation, while addition of the sulfate reduction inhibitor molybdate could reduce Hg methylation in sulfate-limited environments where SRB act as the active Hg methylating microbes. Thus, addition of sulfate as a stimulator and/or molybdate as an inhibitor of sulfate reduction has been an efficient way to identify SRB as active methylators (Bailey et al., 2017; Compeau & Bartha, 1985; Gilmour, Henry, & Mithchell, 1992; Yu et al., 2012). Background sulfate concentrations in environmental matrices usually provide a guide in selecting the dose of molybdate in inhibition assays, or of sulfate in stimulation assays, in experiments designed to distinguish the major microbial guilds that methylate Hg. This consideration has been frequently ignored in some studies. Different amendment levels of molybdate changed the effects of inhibition on Hg methylation (Chen, Bonzongo, Lyons, & Miller, 1997), and thus, to avoid a biased assessment, the proper addition level of molybodate should be at an equimolar concentration with the ambient sulfate level (Fleming et al., 2006; Oremland & Capone, 1988). However, studies showed that by designing the inhibition assays to include multiple levels of molybdate (or sulfate), ranging from 2 to 5 times the ambient sulfate level, the dose effects of the inhibitor (or stimulator) could lead to a better identification of roles of SRB in Hg methylation (Gilmour et al., 1992; Yu et al., 2012). For instance, additions of sulfate at higher than ambient concentration would increase or at least maintain the enhanced methylation rates of SRB as active in situ

methylators, while similar additions of molybdate as an inhibitor would further decrease or keep the reduced rates of methylation. This approach would further determine the effects of the stimulator or inhibitor when added at two or multiple levels to identify potential overdose issues related to the amended reagents (Chen et al., 1997; Yu et al., 2012).

Sulfide is the final reduction product of sulfate metabolism by SRB, and its concentration reflects historic changes in sulfur loading and/or sulfate respiration in the environment (Bailey et al., 2017). Low sulfide concentrations facilitate the formation of neutral mercury sulfide complexes, which could stimulate a faster transport through cell membranes and thus promote methylation of Hg. Higher sulfide concentrations cause the complexation of Hg(II) with sulfide and a reduced Hg bioavailability (Benoit, Gilmour, Mason, & Heyes, 1999). Thus, sulfide affects the speciation and bioavailability of inorganic Hg(II). However, more recent findings suggest that in addition to passive diffusion of Hg into cells (Regnell & Watras, 2019; Schaefer et al., 2011), facilitated transport (Golding et al., 2002) might take place affecting bioavailability and rates of methylation. Sulfide could change the solubility and partition of MeHg into porewater and therefore it serves a dual role, as a ligand for inorganic mercury which mostly decreases Hg(II) bioavailability, and as a ligand with methylmercury which increases MeHg partitioning into porewater (Bailey et al., 2017; Hsu-Kim et al., 2018). Since iron can complex with and scavenge porewater sulfides by forming stable iron–sulfur compounds (Bravo & Cosio, 2020), the concentration of Fe(II) is also a key to MeHg production and its partitioning in the sulfate-impacted freshwater sediment (Bailey et al., 2017). Rather than measuring sulfate concentrations, sulfite and sulfate reduction rates more directly reveal respiratory activities of SRB in a given habitat, and thus represent a better link between SRB and their roles in methylation (Correia & Guimaraes, 2017; King, Saunders, Lee, & Jahnke, 1999). Because Hg(II) has a high affinity to reduced sulfur and thiols are abundant in organic matter, organic matter is strongly correlated with precipitation of metal sulfides and thus, availability of Hg(II) to methylating bacteria. Thus, the complexation model of Hg − S − DOM which considers both factors of reduced sulfur and DOM might better estimate the speciation and bioavailability of Hg(II) than the Hg-S model (Hsu-Kim et al., 2013).

### *2.2.2 Natural organic matter*

Natural organic matter (NOM) acts both as a chemical ligand and as a carrier for Hg(II) and MeHg (Hsu-Kim et al., 2018), and serves as a growth

substrate to microorganisms. The dual roles of NOM strongly influence the speciation and availability of Hg(II) and MeHg, as well as Hg methylation activities in aquatic environments (Khwaja, Bloom, & Brezonik, 2010; Skyllberg, 2008).

Natural organic matter possesses a high affinity to Hg species. Chelation of inorganic Hg(II) with NOM could reduce the bioavailability of Hg for methylation (Bravo et al., 2017; Drott, Lambertsson, Bjorn, & Skyllberg, 2007; Kim, Han, Gieskes, & Deheyn, 2011; Mazrui, Jonsson, Thota, Zhao, & Mason, 2016). In oxic conditions, Hg-NOM complexation might increase Hg(II) partitioning into the solid phase and reduce its availability for methylation. Under low sulfide conditions, complexation of Hg(II) with sulfide could form a nano-particulate form, Hg-S. When testing with strain ND132, Hg-S complexes might have enhanced Hg methylation (Graham, Aiken, & Gilmour, 2013; Graham et al., 2017). Eutrophication is a major environmental response to an increased addition of organic matter, enhancing the extent and duration of anoxic conditions and water column stratification. Previous studies indicated that changed redox gradients were considered major factors causing increased MeHg production (Driscoll et al., 1995; Eckley & Hintelmann, 2006; Merritt & Amirbahman, 2008; Watras, Morrison, Host, & Bloom, 1995). By studying the influences of dissolved organic matter (DOM) on methylation by *G. sulfurreducens* PCA, an iron reducer, and by *P. mercurii* ND132$^{T}$, a sulfate reducer, Zhao et al. (2017) reported that DOM greatly enhanced Hg methylation by the SRB but inhibited Hg methylation by the iron reducer. Therefore, the effects of DOM on Hg methylation were likely bacterial-strain specific, depending on the DOM:Hg ratio and other site-specific conditions. Considering the difficulty of determining NOM composition under different redox regimes, the overall complexation mechanisms of NOM with Hg(II) and MeHg are variable, complex, and still remain largely unknown.

Organic matter in natural environments may be degraded to a variety of substances, e.g., monosaccharides or small degradation products of lipids and proteins, which are further degraded to intermediate metabolites such as volatile fatty acids (VFA), lactate, pyruvate, acetate, and others. These substances might enhance growth of Hg methylating microbes, and thus likely increase Hg methylation activities by stimulating microbial activities (Bravo et al., 2017), increasing energy production (Yu, Reinfelder, et al., 2018), providing methyl groups for methylation, and releasing Hg(II) from cinnabar (Paranjape & Hall, 2017). DOM may not only serve as shuttle molecules for Hg uptake, but also alter cell wall properties that facilitate the first

steps toward Hg(II) internalization (Chiasson-Gould, Blais, & Poulain, 2014). One study showed that phytoplankton-derived organic compounds increased Hg methylation rates in boreal lake sediments through an overall increase of bacterial activity, whereas sediments dominated by terrigenous organic matter inputs led to lower methylation rates (Bravo et al., 2017). In 10 major lakes in China, algae settlement and decomposition after algal blooms enhanced MeHg levels by 54–514% in sediments, mainly due to the stimulated abundances of microbial methylators, rather than due to changed Hg speciation in sediments (Lei, Nunes, Liu, Zhong, & Pan, 2019). After amending sediment slurries with short-chain fatty acids, alcohols, or polysaccharides, Christensen et al. (2018) indicated that amendments of lactate, ethanol, and methanol only led to a minimal increase in Hg methylation rates, while addition of cellobiose caused a drastic decrease in methylation rates, with an associated shift in the microbial community to mostly nonmethylating Firmicutes. Therefore, the molecular composition of organic matter likely determines the nature of its influence on microbial methylation potentials.

### *2.2.3 Iron (Fe)*

Iron in the environment may exist in either ferrous [Fe(II)] or ferric [Fe(III)] oxidation states which are traditionally analyzed by the ferrozine assay (Lovley & Phillips, 1987). The reduction of ferric iron by iron reducing bacteria (FeRB) is a key biogeochemical process influencing microbial Hg methylation (Kerin et al., 2006; Yu et al., 2012). As the reduction potential of Fe(III)/Fe(II) is higher than other electron pairs, Fe(III) reducers can conserve energy by oxidizing a variety of organic compounds and metals. In sulfate-rich habitats, Fe(III) and sulfate reductions can occur concurrently and form ferrous sulfide (FeS) precipitates (Ahmed & Lin, 2017). Addition of Fe(II) thus might reduce sulfide activity, decrease Hg(II) bioavailability as FeS both sorbs and reduces Hg(II) (Bone, Bargar, & Sposito, 2014), and inhibit Hg methylation (Mehrotra, Horne, & Sedlak, 2003; Mehrotra & Sedlak, 2005).

The role of FeRB in Hg(II) methylation in the environment is difficult to be directly distinguished, as is possible for SRB and methanogens, since there is no specific inhibitor of Fe(III) reduction. Since most Fe(III) compounds are highly insoluble, the reduction of Fe(III) might be largely inhibited by other anaerobes including denitrifiers, SRB and methanogens in sediments (Ahmed & Lin, 2017). However, FeRB could directly interact with the solid surface and readily reduce Fe(III) compounds (Lovley & Walker, 2019).

Based on microbial reduction rates, the utilization sequence of ferric compounds by FeRB in anaerobic respiration is likely $FePO_4 \cdot 4H_2O >$ $Fe(OH)_3 > \gamma$-FeOH > $\alpha$-FeOH > $Fe_2O_3$. Fe(III) in less crystalline or amorphous compounds is considered to be more available due to their larger surface areas and higher solubility in sediment or soils (Munch & Ottow, 1983), while chelated Fe(III) ions (e.g., Fe[III]-NTA, Fe[III]-citrate) are mostly used for bacterial culturing and have little relevance to environmental conditions. Based on the in situ levels of Fe(III), freshly synthesized amorphous $Fe(OH)_3$ added at 0.5, 1, and 2 times than the ambient sediment levels significantly stimulated Hg methylation (Yu et al., 2012). Since the solution of $FeCl_3$ is strongly acidic and Fe(III) in the liquid state could be reduced by other reducers such as SRB and denitrifers, direct addition of $FeCl_3$ might cause controversial results especially when the amendment dose is not environmentally relevant, with no consideration of the in-situ Fe(III) level in the study sites. For instance, amendment of $FeCl_3$ stimulated Hg methylation at a lower dose, while higher levels significantly inhibited methylation in mangrove samples (Correia & Guimaraes, 2017). It seems that amorphous $Fe(OH)_3$ serves as a better choice as a Fe(III) amendment reagent. $Fe(OH)_3$ can be synthesized by the method introduced by Cornell and Schwertmann (2003). Therefore, electron acceptor additions of amorphous $Fe(OH)_3$ (Yu et al., 2012), chelated Fe(III) (e.g., Fe(III) citrate) (Gilmour et al., 1998), or FeOOH (Hu et al., 2020; Wu et al., 2020) to stimulate Fe reduction are all suited to partially identify the roles of FeRB in microbial Hg methylation in the natural environment.

### *2.2.4 Inorganic nutrients*

The type and concentrations of inorganic nutrients are one of the key abiotic factors affecting Hg cycling and Hg methylation in natural ecosystems both through biogeochemical interactions and by affecting microbial processes of methylating and non-methylating microbes. Although Hg methylation mechanisms and processes are highly complicated, manipulations to reduce MeHg production are possible by enhancing oxidant capacity, regulation of redox potentials, suppression of Hg methylating microbes, or by other strategies. For example, MeHg abatement was achieved by the addition of nitrate to contaminated Onondaga Lake, NY, USA, to change the redox in the lake and to stimulate denitrifiers which outcompeted methylating anaerobes such as SRB (Beutel et al., 2016; Todorova et al., 2009).

Hypolimnetic zones in stratified lakes and reservoirs are largely anoxic and could be an important source of MeHg. In a whole lake manipulation,

injection of liquid calcium nitrate decreased the maximum hypolimnetic concentrations of MeHg by 94% in Onondaga Lake (Matthews et al., 2013). However, this treatment could be complicated by the multitude of processes that were triggered when nitrate was added, as documented in the hypolimnion of a mesotrophic lake in Minnesota (Austin, Scharf, Carroll, & Enochs, 2016). The addition of liquid calcium nitrate elevated redox and moderately suppressed MeHg production, but once the nitrate was depleted MeHg concentrations increased by almost sixfolds. The authors showed that the hypolimnetic nitrate amendment led to the oxidation of sulfides, accumulation of sulfate, and stimulation of SRB. Therefore, the study proposed that a small and frequent dosing with calcium nitrate was a reasonable management method keeping MeHg concentrations down while regularly monitoring hypolimnetic redox in order to maintain a balance of nitrate and sulfate reductions (Austin et al., 2016). Oxygenation in hypolimnetic waters in reservoirs (McCord, Beutel, Dent, & Schladow, 2016) and lakes (Dent, Beutel, Gantzer, & Moore, 2014) repressed MeHg buildup in bottom waters, while the approach did not substantially decrease mercury levels in fish. Other amendments might include addition of selenite ($SeO_4^{2-}$) which likely inhibits Hg methylation by a similar mechanism as nitrate, and the biodilution of MeHg in fish caused by the eutrophication in natural lakes (Hsu-Kim et al., 2018; Pickhardt, Folt, Chen, Klaue, & Blum, 2002). However, a recent study used microcosms to show that nitrate-amendments resulted in the highest number of hub taxa (key species) and corresponded with enhanced Hg methylation potentials, and that Hg methylation potentials were positively correlated with numerous bacterial families that did not contain the genes specifying methylation, *hgcAB* (Carrell et al., 2021). Therefore, the suppression of MeHg production by using inorganic nutrient amendments is complicated and requires additional investigations.

## 2.3 Previously-recognized and newly-discovered Hg methylating microbes

The Hg methylation phenomenon in the environment was first noted by Jensen and Jernelöv (Jensen & Jernelov, 1969), who showed that MeHg concentrations in lake sediment significantly increased when incubated with Hg(II). Based on loss of activity upon sterilization, the authors proposed that MeHg might be synthesized by microorganisms. It was subsequently confirmed that Hg methylation in sediments and soils was generally of biological rather than chemical nature (Berman & Bartha, 1986). Several methylating

strains were later isolated and cultivated in the laboratory and their Hg methylation activities were confirmed (Choi et al., 1994b; Gilmour et al., 2011). Recent progress in Hg methylation research, triggered by the seminal discovery of the genes that specify the methylation reaction, *hgcAB* (Parks et al., 2013), has resulted in the identification of variety of new Hg methylators as all *hgcAB*-carrying strains that were tested were confirmed as methylators (Gilmour et al., 2013). The relative contribution of different microbial guilds to Hg methylation, critical to understand Hg methylation pathways in natural environments, has not been well studied. Here, we review Hg methylating communities which include four major microbial guilds, SRB, FeRB, methanogens, and fermenters, as well as others. Our discussion is largely based on the concatenated proteins phylogenies of HgcAB. Establishing such phylogenies is likely a more conservative approach that eliminates uncertainties derived from various gene detection-only studies (Gabaldon, 2005; Gadagkar, Rosenberg, & Kumar, 2005).

### *2.3.1 Mercury methylating sulfate reducing bacteria (SRB)*

SRB have been the first to be confirmed as Hg methylating microbes, distinguished by using environmental incubations with molybdate, a specific metabolic inhibitor of sulfate reduction, and 2-bromoethane sulfonate (BES), an inhibitor of methanogenesis (Compeau & Bartha, 1985). This early study showed that sulfate reducing bacteria (SRB) in saltmarsh sediments were responsible for 95% of the Hg methylation, while methanogens did not synthesize MeHg. The nearly complete inhibition of Hg methylation by molybdate in freshwater reservoir sediments further supported the hypothesis that SRB were responsible for Hg methylation (Gilmour et al., 1992). Currently, it is generally believed that SRB might play the dominant role in sulfate-replete habitats such as estuarine and marine environments. In low-sulfate and sulfate-limited freshwater sediments, sulfate loading increases the production of MeHg (Jeremiason et al., 2006; King, Kostka, Frischer, & Saunders, 2000; Yu et al., 2012), while a reduction in sulfate loading decreases MeHg production and bioaccumulation (Orem et al., 2020). In freshwater ecosystems, SRB could methylate Hg in absence of sulfate by forming syntrophic interactions and by fermentation (Gilmour et al., 2011; Pak & Bartha, 1998a; Yu, Reinfelder, et al., 2018). However, SRB may not be the primary methylators in river sediments (Fleming et al., 2006; Yu et al., 2012), rice paddies (Liu et al., 2018), and boreal lake sediments (Bravo et al., 2018).

Isolation of Hg methylating SRB strains and further Hg methylation assay experiments have initiated and facilitated the study of Hg methylation mechanisms. Two early model SRB isolates were *Desulfovibrio desufuricans* LS (Compeau & Bartha, 1985) and *D. desulfuricans* ND132 (Gilmour et al., 2011), now reclassified and renamed *Pseudodesolfovibrio mercurii* ND132$^T$ (Gilmour et al., 2021); regretfully, strain LS was later lost. Both were isolated from estuarine sediments. The commonality of the genera *Desulfovibrio* and *Pseudodesulfovibrio* among methylators is due to the high Hg methylation capability of these genera (Gilmour et al., 2011) and their high abundance in sulfate reducing communities (Muyzer & Stams, 2008). Based on studies with strain LS, Bartha and his group first proposed a metabolic pathway and basic mechanism of Hg methylation in the 1990s (see above) (Choi & Bartha, 1993; Choi et al., 1994a, 1994b).

The SRB guild is highly diverse with members unlikely to be phylogenetically affiliated with each other (Waite et al., 2020). In addition to the dominant Hg methylators included in the family *Desulfovibrioaceae* (Gilmour et al., 2011), other confirmed Hg methylating SRB include species from *Desulfomicrobiaceae*, *Desulfobacteraceae*, *Desulfobulbaceae*, *Desulfohalobiaceae*, *Desulfuromanadaceae*, and *Peptococcaceae* (Ekstrom et al., 2003; Gilmour et al., 2013; King et al., 2000; Ranchou-Peyruse et al., 2009).

After the *hgcAB* genes were discovered in strain ND132$^T$ and *G. sulfurreducens* PCA (Parks et al., 2013), explorations by PCR detection and sequencing of the *hgcAB* genes from different natural habitats have shown an extended diversity of previously unknown *hgcAB*-carrying Hg methylating microbes including SRB (Peterson et al., 2020; Podar et al., 2015; Villar, Cabrol, & Heimburger-Boavida, 2020). However, the detected operational taxonomic units (OTUs) in environmental gene assemblages could not be related to Hg methylation capabilities, or lack thereof. Thus, an overestimating risk of novelty and diversity of Hg methylating microbes may emerge from investigations that solely relay on culture-independent approaches.

To explore the expanded diversity and novelty of Hg methylators, we have searched all available HgcA and B proteins of SRB ($HgcAB_{SRB}$) at NCBI by using the HgcA and B amino acid sequences of strain ND132$^T$ as the query. The two proteins specifying Hg methylation activities of each potential Hg methylating species were concatenated. The fused HgcAB sequences of $HgcAB_{SRB}$ were first aligned and their ends were trimmed by Jalview to make the sequences more comparable for further phylogenetic analyses (Waterhouse, Procter, Martin, Clamp, & Barton, 2009).

The best amino acid substitution model of the sequences for the maximum likelihood phylogeny analyses was optimized and tested by MEGA (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). By selecting the matrix name of LG and the Substitution Model of PROTGAMMAI (ML + rapid bootstrap at 1000 replicating), the phylogeny of $HgcAB_{SRB}$ sequences was analyzed by using GUI RAxML (Kozlov, Darriba, Flouri, Morel, & Stamatakis, 2019). An amino acid phylogeny was established for SRB (Fig. 2), with the same phylogenetic analysis conducted for the iron reducing bacteria and methanogens (see below). SRB families containing HgcAB included in the tree are *Desulfovibrionaceae* (e.g., *Desulfovibrio* spp., *Pseudodesulfovibrio* spp., *Desulfobaculum xiamenense*) together representing 28% in the total $HgcAB_{SRB}$ data base, *Desulfobacteraceae* (*Desulfobacula phenolica*, *Desulfococcus multivorans, Desulfosarcina alkanivorans*, *Desulfobacterium vacuolatum*, *Desulfospira joergensenii, Desulfobacula phenolica, Desulfotignum balticum, Desulfamplus magnetovallimortis, Desulfoluna spongiiphila*), *Desulfobulbaceae* (*Desulfobulbus mediterraneus, D. propionicus, D. japonicas, Desulfofustis glycolicus, Desulfopila aestuarii, Desulforhopalus* spp., *Desulfotalea* spp.), *Desulfonatronaceae* (*Desulfonatronum thioautotrophicum, D. thiodismutans, D. lacustre*), *Desulfomicrobiaceae* (*Desulfomicrobium escambiense*, *D. baculatum*, *D. norvegicum*, *D. apsheronum*), *Desulfohalobiaceae* (*Desulfonatronovibrio hydrogenovorans, Desulfonatronospira thiodismutans*), *Desulfuromonadaceae* (*Desulfuromonas soudanensis*), *Dissulfurirhabdaceae* (*Dissulfurirhabdus thermomarina*), *Syntrophobacteraceae* (3.3% of the total), and *Syntrophaceae* (*Syntrophus*) from Proteobacteria. Several previous Hg methylating *Desulfovibrio* species such as *D. aespoeensis*, *D. indicus*, *D. profundus* have been reclassified into the new genus *Pseudodesulfovibrio* (Cao et al., 2016) as is the case for strain $ND132^T$ (see above) (Gilmour et al., 2021), while *D. africanus* has been regrouped as *Desulfocurvibacter africanus* (Spring et al., 2019).

It is interesting to note that fused HgcAB sequences vary largely even within *Pseudodesulfovibrio* spp. Some fused HgcAB are sometimes observed in genomic sequences but, to the best of our knowledge, might lack methylation activity (Podar et al., 2015). Phylogenetic analyses of HgcAB generate various fragmental clusters with respect to genome lineages, in the tree, likely revealing evolution by horizontal gene transfer consistent with other reports (Gionfriddo et al., 2020). Other novel families containing potential Hg methylators are *Peptococcaceae* (*Desulfitibacter* spp., *Desulfitobacterium metallireducens*), *Sporomusaceae (Acetonema longum*), *Ruminococcaceae* (*Ethanoligenens harbinense*), and *Syntrophomonadaceae* (*Dethiobacter alkaliphilus*) from the phylum *Firmicutes*, and *Nitrospira* from the phylum *Nitrospirae*.

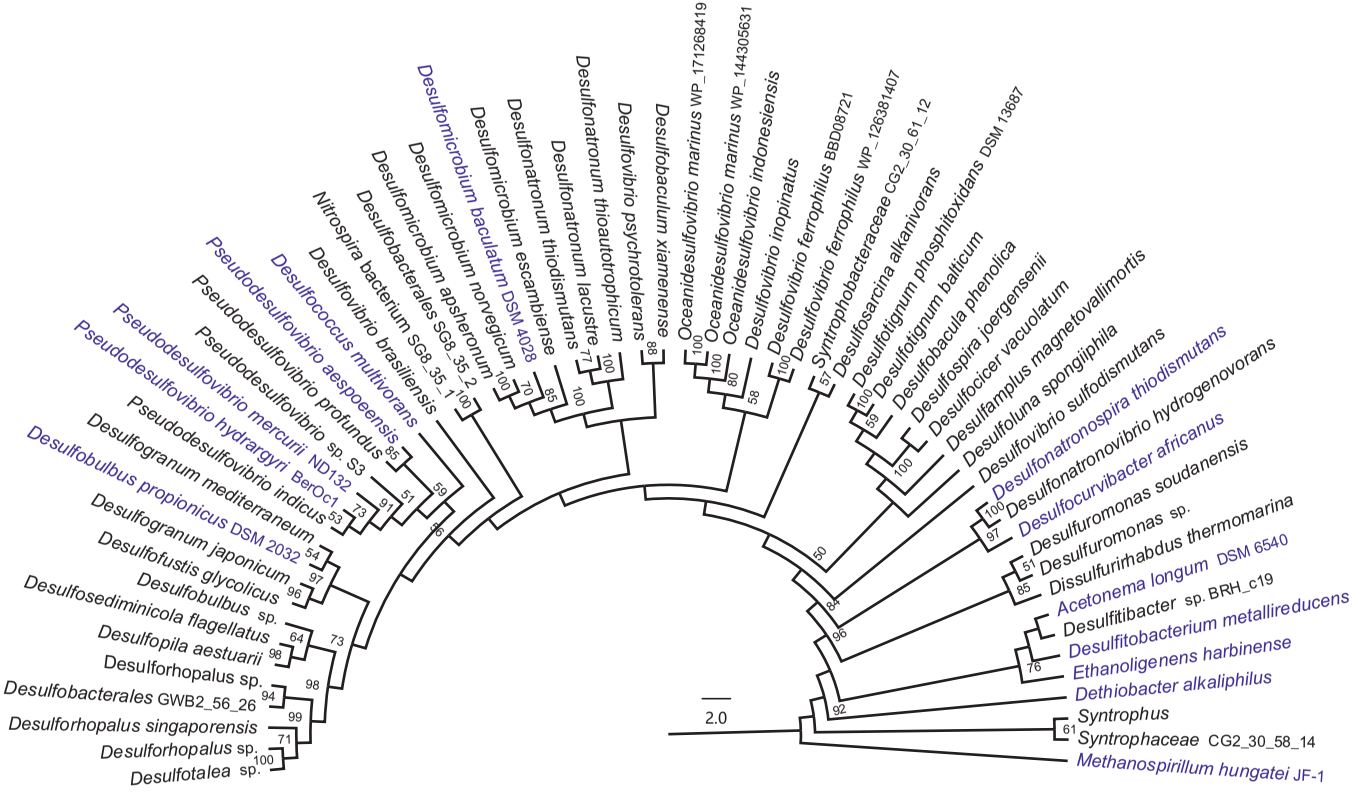


**Fig. 2** A maximum likelihood phylogeny of fused HgcAB obtained from the genomes of SRB and related species. Sequences belonging to experimentally confirmed methylators are highlighted by bold (in the print version) or blue (in the online version) color. Numbers on the tree represent bootstrap values.

### 2.3.2 Mercury methylating iron reducing bacteria (FeRB)

Iron reducing bacteria are another major group contributing to MeHg synthesis in aquatic ecosystems (Fleming et al., 2006; Kerin et al., 2006). Due to the lack of an efficient specific inhibitor of iron reduction, it is hard to document a role of FeRB in Hg methylation activities. Nevertheless, early reports that focused on benthic river or lake habitats suggested methylation by FeRB by showing stimulated MeHg synthesis upon Fe(III) amendments (Warner, Roden, & Bonzongo, 2003; Yu et al., 2012). Moreover, new evidence has since shown that *Geobacteraceae* and other FeRB may be a major group of Hg methylators having a dominant role in microbial MeHg production in rice paddy soils (Liu et al., 2018), boreal forest soils (Xu et al., 2019), lake sediments (Bravo, Peura, et al., 2018; Bravo et al., 2018), and boreal wetlands and peatlands (Schaefer, Kronberg, Bjorn, & Skyllberg, 2020). To the best of our knowledge, Hg methylation has not been reported among iron reducing Archaea.

The FeRB including *G. sulfurreducens* PCA, *Geobacter metallireducens* GS-15, and *Geobacter* sp. strain CLFeRB were shown early to be active Hg methylators. From the phylogenetically clustering analyses of 16S rRNA genes, these strains are affiliated with most Hg methylating SRB and syntrophs within the same class *Deltaproteobacteria*, but remain different at the domain level from methanogens (Kerin et al., 2006; Ranchou-Peyruse et al., 2009; Yu et al., 2012). We note that the *Deltaproteobacteria* have been newly classified to the new phylum *Desulfobacterota* by Waite et al. (2020). However, phylogenetic comparisons of HgcA revealed that, the sequence of the functional protein HgcA of *G. sulfurreducens* PCA is 43.2% similar to that of the archaeon *M. hungatei*, which is the same as that with *P. mercurii* ND132$^{T}$ (43.2%). The similarity of HgcB in PCA with *M. hungatei* (49.4%) is even higher than its similarity (44.9%) with *P. mercurii* ND132$^{T}$. HgcA of *M. hungatei* is more similar with that of *Syntrophus* (51.3%) and *Syntrophaceae* (46.9%). The similar phylogenetic correlations among FeRB, SRB and methanogens are shown in Fig. 2 (HgcAB of SRB) and 3 (HgcAB of FeRB). Overall, concatenated HgcAB of FeRB showed much closer phylogenetic affiliations with those of SRB and methanogens as compared with their 16S rRNA gene relationships (Gilmour et al., 2013; Yu et al., 2012, 2013), supporting observations in other studies that *hgcAB* genes might evolve by horizontal gene transfer (Gionfriddo, Wymore, et al., 2020; Podar et al., 2015; Villar et al., 2020).

From the phylogeny of concatenated HgcAB of FeRB (Fig. 3), FeRB with a potential for Hg methylation consist mostly of the genera *Geobacter*

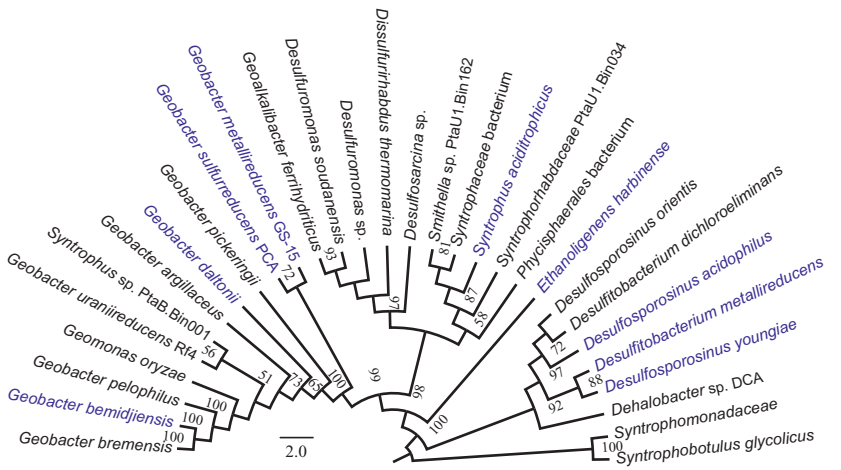


**Fig. 3** Fused HgcAB phylogeny of FeRB and related species. Experimentally confirmed Hg methylators are highlighted by bold (in the print version) or blue (in the online version) color. Numbers on the tree represent bootstrap values.

spp., the *Desulfuromonas* spp.-related cluster, and the syntroph cluster of the order *Syntrophobacterales*. Members of *Geobacter* spp., family *Geobacteraceae*, and *Desulfuromonas* spp., family *Desulfuromonadaceae*, are closely related species in the class *Desulfuromonadales* and could conduct Fe(III) reduction (Badalamenti, Summers, Chan, Gralnick, & Bond, 2016; Roden & Lovley, 1993; Wilkins, Livens, Vaughan, & Lloyd, 2006). Multiple species from the *Geobacteraceae* are confirmed as Hg methylators, although not all FeRB methylate Hg, e.g., *Shewanella* strains from *Gammaproteobacteria* (Gilmour et al., 2013; Kerin et al., 2006). Some species from the *Desulfuromonas* cluster together with most species from the cluster of syntrophs (Fig. 3) are also SRB which might or might not perform Fe(III) reduction.

Taxa in the fourth group of the tree on the right side are mostly affiliated with the family *Peptococcaceae*-related species (e.g., *Dehalobacter*) of the *Clostridiales* from the Firmicutes (Fig. 3). Peptococcaceae is taxonomically heterogeneous, represented by *Syntrophobotulus* spp., *Dehalobacter* spp., and *Desulfitobacterium* spp. All members of the *Peptococcaceae* are obligate anaerobes, and could act as chemoorganotrophs, chemolithoheterotrophs, chemolithoautotrophs, or syntrophs (with hydrogenotrophs) by using flexible metabolic pathways (Stackebrandt, 2014). Previous studies indicated that isolates from the *Peptococcaceae* could conduct iron- (Kunapuli et al., 2010) or sulfate (Winderl, Penning, Netzer, Meckenstock, & Lueders, 2010) reductions, while degrading toluene. Several species are confirmed

as Hg methylators, including *Desulfosporosinus youngiae*, *D. metallireducens*, *Desulfitobacterium dehalogenans*, *Desulfosporosinus acidophilus*, and *E. harbinense* (Gilmour et al., 2013). The methylating strain *D. dehalogenans* is not included in the tree since only a partial sequence of HgcA from this strain is available.

### 2.3.3 Mercury methylating methanogens

Methanogens were initially proposed as Hg methylators in late 1960s, because cell free extracts of *Methanobacterium bryantii* generated MeHg when spiked with Hg(II) (Boone, 1987; Bryant et al., 1967; Wood et al., 1968). This observation was largely ignored later when SRB were identified as the principal Hg methylators in a saltmarsh (Compeau & Bartha, 1985) and pure cultures of one methanogen failed to methylate (Pak & Bartha, 1998c). However, using specific metabolic inhibitors and stimulators, Hamelin et al. (2011) were the first to show that Hg methylation in lake periphytons was attributed to methanogens. Realizing that $Na_2S$ may inhibit methylation (Yu, 2011), Yu et al. (2013) replaced $Na_2S$ with $TiCl_3$ as a reducing agent in the methanobacterium medium (DSMZ 119), and clearly showed that *M. hungatei* JF-1 (DSM 864) methylated Hg at rates and yields similar to those previously reported for SRB and FeRB. This study further proposed methanogens as a new guild of Hg methylators (Yu et al., 2013). The inhibitory effect of sulfide in the reducing agent may explain why some previous studies had failed to detect Hg methylation by Hg methylating methanogens. Moreover, following the discovery of *hgcAB* (Parks et al., 2013), several putative Hg methylating methanogens were identified (Gilmour et al., 2013) and later confirmed (Gilmour et al., 2018).

Blast searches found that all orthologs of HgcAB in the genomes of methanogens are affiliated with the phylum *Euryarchaeota*, while only analogs of HgcAB are present in some genomes representing the phylum *Crenarchaeota* (see Fig. 4). A total of 51 concatenated HgcAB were found in the genomes of either pure strains or from environmental samples by de novo genomic assembly. Within the 51 HgcAB orthologs in *Euryarchaeota*, 88.2% were from the class *Methanomicrobia* (74.5% from the order *Methanomicrobiales*, 5.9% *Methanocellales*, and 7.8% *Methanosarcinales*). The remaining 9.8% of the 51 orthologs were from the order *Methanomassiliicoccales* of the class *Thermoplasmata* (Fig. 4). The nine confirmed Hg methylating methanogens included seven strains of *Methanomicrobiales*, one *Methanocellales*, and one *Methanomassiliicoccales* (Gilmour et al., 2018, 2013; Yu et al., 2013). Intriguingly, the genome

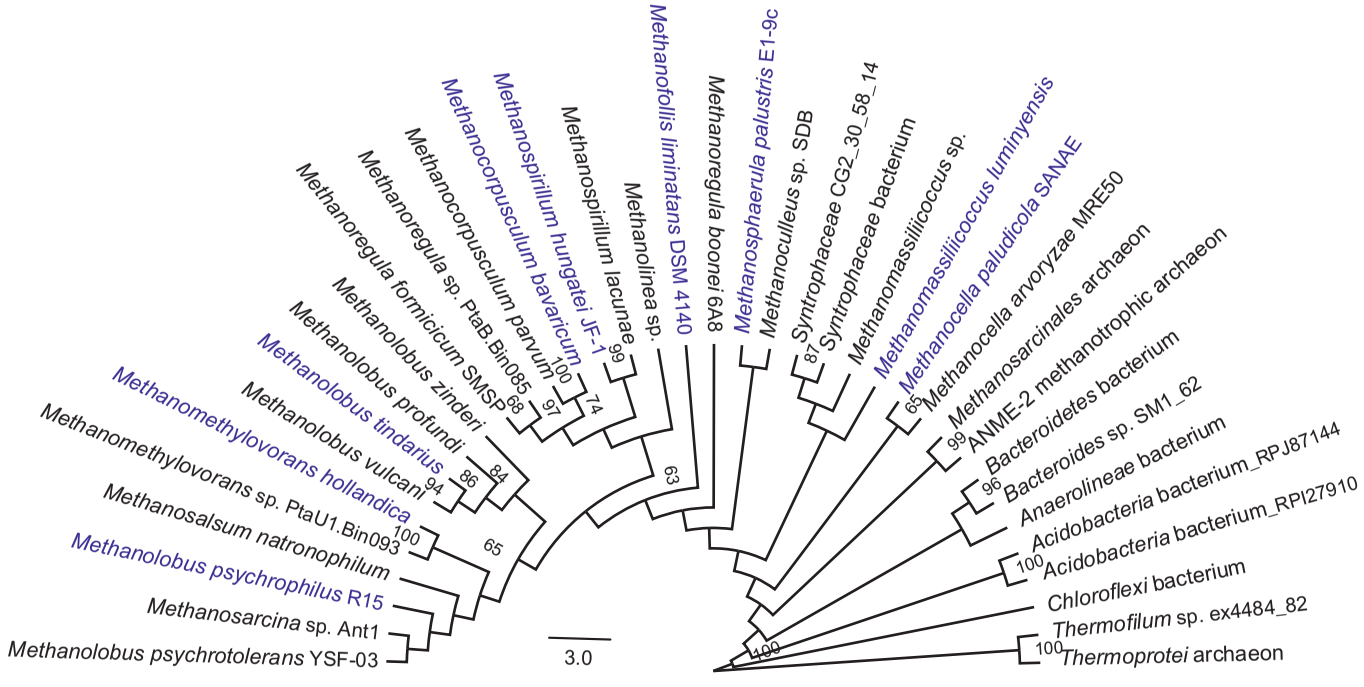


**Fig. 4** Fused HgcAB phylogeny of methanogens and related species. Experimentally confirmed Hg methylators are highlighted by bold (in the print version) or blue (in the online version) color, with *Thermofilum* as the outgroup. Numbers on the tree represent bootstrap values.

of *M. bryantii*, used by Wood et al. (1968) in the first demonstration of Hg methylation by cell extracts, does not contain either orthologs or paralogs of HgcA and HgcB. It is possible that (1) the archaeon does not methylate Hg even though a cell extract did (Wood et al., 1968), (2) Hg methylation by *M. bryantii* might occur by a different biochemical pathway, or (3) methylation may be mediated by genes with low homology with *hgcA* and *hgcB* (Yu et al., 2013).

### 2.3.4 Mercury methylating syntrophs

By forming a tightly coupled mutualistic metabolism, microbial syntrophs are crucial for degradation of natural polymers in anaerobic habitats. Syntrophs classically include two partners: the syntrophic primary fermenter which degrades intermediate substrate to small molecules, and the consumer which mineralizes the fermented products (Morris, Henneberger, Huber, & Moissl-Eichinger, 2013). In anaerobic environments low in electron acceptors, fermenting bacteria are the major organisms degrading natural organic polymers including polysaccharides, proteins, nucleic acids, and lipids to intermediate products such as acetate and longer-chain fatty acids (e.g., propionate), alcohols, $CO_2$, formate, and $H_2$. Syntrophs then further metabolize these intermediate products to $H_2$, formate, and acetate, and cross-feed them to hydrogenotrophic or acetotrophic methanogens to form methane ($CH_4$) and carbon dioxide (McInerney, Sieber, & Gunsalus, 2011; McInerney et al., 2008). Syntrophic interactions between fermenting and hydrogenotrophic microbes may be of particular importance to the microbial production of MeHg in sulfate-limited freshwater, or organic carbon-limited brackish environments (Yu, Reinfelder, et al., 2018). Therefore, when assessing methylation activities in the environment, direct prediction based only on laboratory tests of pure cultures may be misleading. Methylation stimulated by the interaction between syntrophs and methanogens suggests that understanding Hg methylation within the context of the complexity of microbial interactions is crucial to unravel methylation in natural habitats (Yu, Reinfelder, et al., 2018).

Although syntrophs are highly diverse in terms of their taxonomic affiliation, most species known so far are from the phylum *Desulfobacterota* (Waite et al., 2020) and the class *Clostridia* of the phylum *Firmicutes*. Common genera of syntrophs from the *Desulfobacterota*, acting as the syntrophic primary degraders, include *Syntrophobacter*, *Syntrophorhabdus*, *Syntrophus*, *Smithella*, *Desulfovibrio*, *Desulfoglaeba*, *Geobacter*, and *Pelobacter*. The order *Syntrophobacterale*s contains three families, the *Syntrophobacteraceae*, *Syntrophorhabdaceae*, and *Syntrophaceae*.

Most species from these families tightly couple with methanogens during propionate degradation, but they could also grow alone by respiring sulfate (McInerney et al., 2008; Wallrabenstein, Hauschild, & Schink, 1994). These bacteria are usually less competitive or have low sulfate reduction capabilities in comparison with *Desulfovibrio* spp. (Muyzer & Stams, 2008; Yu, Reinfelder, et al., 2018), although most of them contain *dsrAB* genes. The assumption is that these microbes have lost the ability to efficiently reduce sulfate during evolution in low-sulfate and/or sulfite in methanogenic environments (Imachi et al., 2006; Plugge, Zhang, Scholten, & Stams, 2011). The presence of *dsrAB* in these bacteria would be a genetic remnant and reflects both an ancient sulfate/sulfite-respiring potentials and an evolutionary connection between the sulfate-reducing and syntrophic lifestyles (Imachi et al., 2006; Plugge et al., 2011). *Syntrophobacter wolinii*, which is affiliated with the order *Syntrophobacterales*, was the first syntrophic propionate oxidizer to be isolated (Boone & Bryant, 1980). This strain has been confirmed as a weak Hg methylator, while two other propionate oxidizers/sulfate reducers syntrophs, *Syntrophobacter fumaroxidans* and *Syntrophobacter sulfatireducens* TB8106 do not methylate (Yu, Reinfelder, et al., 2018). In addition, *hgcAB* homologs were identified in the genomes of *Syntrophorhabdus aromaticivorans* UI (Parks et al., 2013). The family *Syntrophaceae* includes four genera, *Syntrophus, Smithella, Desulfobacca, and Desulfomonile*. Among these, *Syntrophus aciditrophicus* syntrophically metabolizes benzoate, a variety of fatty acids, crotonate, and butyrate with $H_2$-consumers, and is a confirmed Hg methylator (Gilmour et al., 2013). *Smithella propionica* grows with butyrate, malate, and fumarate in coculture with a methanogen, and is another propionate-oxidizing syntroph; some *Smithella* spp. contain *hgcAB* gene homologs (Fig. 3). Both *Syntrophus* and *Smithella* are unable to use sulfate as an electron acceptor, likely missing the *dsr* genes (Plugge et al., 2011).

As a group of classic SRB and dominant Hg methylators, *Pseudodesulfovibrio* spp. are important facultative syntrophs which degrade substrate such as lactate by fermentation when lacking external electron acceptor (sulfate) and may live with or without the association with methanogens (Pak & Bartha, 1998a; Yu, Reinfelder, et al., 2018). *Pelobacter* spp. syntrophically metabolize ethanol, and *P. seleniigenes* contains an *hgcA* homolog (Liu et al., 2018). Methylating FeRB may also partake in syntrophic interactions. For example, by coupling with *Wolinella succinogenes*, methylating *G. sulfurreducens* could oxidize acetate and grow efficiently with nitrate as the electron acceptor in the absence of ferric iron or other electron acceptors.

Slow syntrophic acetate degradation was also possible between *G. sulfurreducens* and *D. desulfuricans* CSN but only with nitrate as an electron acceptor (Cord-Ruwisch, Lovley, & Schink, 1998). While methylation potentials during these interactions have not been reported, these findings show that Hg methylating FeRB and SRB might contribute to MeHg synthesis in the environment using syntrophic pathways (Yu, Reinfelder, et al., 2018), a topic that has been little studied to date.

The syntrophs from the *Clostridia* of the phylum *Firmicutes* mainly belong to the genera *Desulfotomaculum*, *Syntrophobotulus*, *Pelotomaculum* from the family *Peptococcaceae*, *Sporotomaculum* from the family *Desulfallaceae*, and *Syntrophomonas*, *Syntrophothermus*, and *Thermosyntropha* from the family *Syntrophomonadaceae*. *Desulfotomaculum* spp. can live as SRB by using sulfate as electron acceptor, and also syntrophically by metabolizing propionate or aromatic acids in association with methanogens. The *Desulfotomaculum* subcluster Ih consists of syntrophs that may have lost their ability to reduce sulfate (Plugge et al., 2011). *Desulfotomaculum ruminis* was confirmed as a Hg methylator (Kaschak, Knopf, Petersen, Bings, & Konig, 2014). In addition, *hgcA/hgcB* homologs were identified in the genome of *Syntrophobotulus glycolicus* DSM 8271 (Parks et al., 2013). Syntrophic propionate-oxidizing *Pelotomaculum* spp. are *P. schinkii*, *P. thermopropionicum*, and *P. propionicicum*. *Syntrophomonas* spp. syntrophically oxidize fatty acids in a coculture with a hydrogen/formate-consuming microorganism, and usually cannot reduce sulfate (Plugge et al., 2011). Several *Syntrophomonas* spp. might contain homologous or analogous *hgcA* genes (Fig. 3; Lin et al., 2021).

Methanogenic archaea from the *Euryarchaeota* phylum are commonly the consuming partner in syntrophic relationships, efficiently oxidizing major electron donors (e.g., hydrogen and formate) (Morris et al., 2013). At the same time, methanogens depend on fermenting microorganisms for the production of their metabolic substrates, $H_2$, $CO_2$, and acetate (Plugge et al., 2011). The microorganisms involved in mutualistic and synergistic interactions are much more diverse than just the syntrophs and methanogens described here, and might include the majority of known and unknown microbes (Morris et al., 2013). The proposed roles of syntrophy in Hg(II)-methylation have been reported in forest wetlands (Schaefer et al., 2020; Yu et al., 2010), estuarine wetlands (Bae, Dierberg, & Ogram, 2019), and northern peatlands (Hu et al., 2020; Roth et al., 2021). Hu et al. (2020) reported that Hg methylation in peatlands progressed from SRB-dominated metabolism in young mires to methanogenic- and syntrophic-dominated pathways in older peatland systems (Hu et al., 2020).

Roth et al. (2021) showed the increased role of syntrophy as poor fans were converted to rich fans by an input of nutrients. In freshwater systems where sulfate is scarce, the syntrophy between SRB and methanogens is usually energetically favored and may play important roles in MeHg production (Yu, Reinfelder, et al., 2018). In brackish or saline environments where microbial activities may be limited by the availability of oxidizable organic substrate, not by sulfate, syntrophy between *Syntrophobacter* spp. and $H_2$-utilizing SRB (e.g., *Desulfovibrionaceae*) may be established in a propionate-dependent sulfate reduction, or syntrophic fermentation (Liu & Conrad, 2017), and play a dominant role in MeHg production in the ecosystem (Yu, Reinfelder, et al., 2018).

### 2.3.5 Other novel species of mercury methylating prokaryotes

As described in the last section, species from the family of *Peptococcaceae* and *Syntrophomonadaceae*, phylum *Firmicutes*, are important syntrophs and fermentors. As a phylogenetically heterogeneous group which could conduct iron- (Kunapuli et al., 2010) or sulfate (Winderl et al., 2010) reductions, many *Firmicutes* contain HgcAB homologs (Figs. 3 and 4) and some have been confirmed as Hg methylators (Gilmour et al., 2013). Firmicutes also likely form the largest cluster of novel Hg methylating microbes. In light of the importance of fermentation in anaerobic metabolism in many environments and in many industrial applications, the *Firmicutes*, likely the least explored Hg methylators, may be important contributors to MeHg formation. The capability of Hg methylation might be derived from the flexible metabolisms of *Firmicutes*, i.e., in the reduction of both sulfate and iron, fermentation as well as syntrophy. As most sulfate-reducing bacteria, the Firmicutes *Desulfosporosinus* spp. can oxidize $H_2$, lactate, pyruvate, glycerol, glucose, and fructose (*D. acidiphilus* SJ4(T)), degrade toluene (*D. youngiae*), or reduce Fe(III) (*D. meridiei* and *D. orientis*). Some *Desulfosporosinus* spp. can also reduce $NO_3^-$ or As(V) as terminal electron acceptors for growth. *D. acidophilus* and *D. youngiae* are two confirmed Hg methylating SRB, and the methylation genes, *hgcAB,* were also found in *Desulfosporosinus orientis*, and *Desulfosporosinus* sp. OT (Gilmour et al., 2013). *D. orientis* was reclassified from the previous species *Desulfotomaculum orientis* in 1997 (Stackebrandt et al., 1997). As confirmed Hg methylators (Gilmour et al., 2013), *E. harbinense*, family Ruminococcacease, is a $H_2$-ethanol co-producing fermenter (Li et al., 2019), while *D. alkaliphilus* from the family *Syntrophomonadaceae*, can use thiosulfate, elemental sulfur and polysulfide as terminal electron acceptors in respiration (Sorokin, Tourova, Mussmann, & Muyzer, 2008).

Based on the phylogenetic analyses of HgcAB homologs which are related to those of the SRB, FeRB, and methanogens, novel potential Hg methylating species are common (Figs. 2–4). Others have reported novel potential Hg metylating microbes based on the presence of HgcAB homologs in the phylum *Nitrospirae* (Fig. 2) (Baker, Lazar, Teske, & Dick, 2015), and *Planctomycetes* (e.g., *Phycisphaerales*) (Fig. 3) (Zhou et al., 2020). Novel HgcABs were observed in anaerobic methanotrophic archaea (ANME) which were originated from deep sea sulfate-methane interfaces (Fig. 4). ANME allows energy conservation by coupling anaerobic oxidation of methane (AOM) with sulfate reduction by SRB (Yu et al., 2018). Novel HgcAB orthologs were also found in the phylum *Bacteroidetes* from estuarine sediments (Baker et al., 2015), and in the phylum *Chloroflexi* from rice paddies (Liu, Yu, Zheng, & He, 2014) or from deep terrestrial subsurface (e.g., *Anaerolineales* bacterium) (Fig. 4) (Probst et al., 2018). The phylum *Acidobacteria* predominantly includes bacteria that occur wildly in acidic forest and wetland soils around the world, and plays crucial ecological roles in carbohydrate degradation, especially those involved in hemicellulose degradation (de Chaves et al., 2019; Kielak, Barreto, Kowalchuk, van Veen, & Kuramae, 2016). The two HgcAB orthologs as the representatives of this phylum, isolated from Nevada wetland sediments, indicated that *Acidobacteria* might be directly involved in Hg methylation (Fig. 4; (Dalcin Martins et al., 2018)), a finding consistent with previous observations in an Adirondack lake wetland (Yu, 2011). Based on environmental metagenomic studies and metagenome assisted genome (MAG) analyses, the diversity of potential Hg methylating microbes containing *hgcA* or h*gcAB* genes has been further extended to the phyla *Aminicenantes*, *Spirochaetes* (Jones et al., 2019), *Nitrospirae*, *Planctomycetes* (Gionfriddo et al., 2020), *Acidobacteria*, and *Actinobacteria* (McDaniel et al., 2020).

In summary, the potential for microbial Hg methylation is broadly distributed among anaerobic microbial taxa that possess diverse metabolisms and are found in diverse anoxic environments. These observations imply that MeHg is likely formed globally and under varied conditions, resulting in food chain contamination and the potential exposure of humans and wildlife.

## 2.4 Evolutionary and environmental implication of putative *hgcAB* genes

Based on presence of orthologous Hg methylation genes, the number of new species containing hypothetical Hg methylators has grown explosively

in recent years. One recent study has reported ~4500 HgcA homologs from genomes and metagenomes (Cooper et al., 2020). As discussed above, the boundaries of novel potential Hg methylating microbes have been rapidly expanded not only geographically but also phylogenetically, although only few studies (Gilmour et al., 2018, 2013) have tested the Hg methylation capability of these potential methylators.

Previously recognized *hgcAB* genes were mainly distributed sporadically among Proteobacteria (especially from the newly defined phylum *Desulfobacterota* (Waite et al., 2020)), *Firmicutes*, and *Euryarchaeota*. Recent gene studies have further extended *hgcAB* phylogenetic distribution, and indicated that the genes also likely exist in *Chloroflexi* (*Dehalococcoides*), *Chrysiogenetes*, *Nitrospirae*, *Aminicenantes*, *Kiritimatiellaeota, Planctomycetes*, *Verrucomicrobia*, *Chlamydiae*, *Lentisphaerae* (all belonging to the PVC superphylum), *Spirochaetes*, *Elusimicrobia*, and *Acidobacteria* (Cooper et al., 2020; Jones et al., 2019; McDaniel et al., 2020). By analyzing the phylogenetic relationships of Hg methylators among SRB, which contain most of the currently recognized methylating species (Fig. 2), methylating SRB strains are scattered throughout the phylogenetic tree and mixed with putative Hg methylators from other phyla (e.g., *Nitrospirae*) (McDaniel et al., 2020). Within the same genus such as *Desulfovibrio* spp., the concatenated HgcAB are highly disparate and sporadically distributed among different clusters (Fig. 2), indicating that phylogenetically closely related organisms may have different Hg methylation capabilities (Benoit, Gilmour, Heyes, Mason, & Miller, 2003). Hg methylation potentials seem neither genus- nor species-dependent, suggesting that the analysis by metagenomic sequences of methylating communities and subsequent identification at the genus level (e.g., 16S rRNA genes) of methylating members, are not an efficient tool to explore the evolutionary and environmental roles of potential Hg methylators in natural environments (Gionfriddo, Wymore, et al., 2020; McDaniel et al., 2020; Ranchou-Peyruse et al., 2009).

When identifying the *hgcAB* genes, Parks et al. (2013) proposed that the sparse phylogenetic gene distribution of the *hgcAB* system might be due to gene loss or lateral gene transfer (or both) across distant taxa, and that the evolutionary advantage and physiological roles implied from such sporadic distribution across phyla, are unknown. Gionfriddo, Wymore, et al. (2020) indicated that the phylogeny of HgcA sequences is not congruent with species phylogeny, and that metagenome-resolved HgcA sequences tend to cluster by environment. For instance, HgcA proteins from marine metagenomes often form distinct clusters separated from those from freshwater

aquifers. Thus, it seems that horizontal gene transfer (HGT) in environments that support syntrophic interactions by the reductive acetyl-CoA pathway might drive distribution patterns and diversity of methylating microbes (Gionfriddo, Wymore, et al., 2020; Podar et al., 2015). By identifying HgcAB proteins in publicly-available isolate genomes and MAGs, McDaniel et al. (2020) presented nearly 1000 putative bacterial and archaeal Hg methylators spanning 30 phyla from numerous environments. Their study showed that the HgcAB protein phylogeny was incongruent with the species tree phylogeny constructed with concatenated ribosomal proteins, consistent with the results of Gionfriddo, Wymore, et al. (2020). Moreover, *Desulfobacterota* HgcAB sequences clustered with the HgcAB sequences from Actinobacteria, Nitrospirae, Spirochaetes, and members of the PVC superphylum. Though a variety of current studies have shown HGT signatures and habitat-specific phylogenetic clustering patterns of Hg methylation genes, both suggesting environmental selection (Cohan, 2002), the driving force for selection of *hgcAB* genes and their environmental distribution remains largely unknown.

Hg methylation genes have become the direct and most useful biomarkers to link MeHg pollution and microbial Hg methylation activities in studies of Hg contamination. By detecting and sequencing the genes, we could identify potential Hg methylating microbes and their communities, and investigate potentially dominant pathways of MeHg synthesis, thus providing support for further management actions to control MeHg contamination. By quantifying the *hgcAB* gene copy number using primer sets specific for different methylation groups, we could analyze the abundance of Hg methylating microbes, and predict Hg methylation potentials and activity efficiencies in the environment. However, by exploring a range of environmental samples (freshwater, estuarine, and organic-rich sediment systems) in the coastal eastern US, Christensen et al. (2019) recently showed that there were no strong correlations between the amount of *hgcAB* genes and total Hg and MeHg concentrations. Thus, challenges remain if we are to evaluate ambient MeHg concentrations based only on measuring *hgcAB* gene abundances and methylation rates. Considering that in situ MeHg concentrations in specific environments represent an overall net process, an extensive prediction model which integrates multiple parameters such as microbial Hg methylation rates, MeHg degradation rates, hydraulic exchanges, biogeochemical absorption and complexation, and other factors (Kwon, Selin, Giang, Karplus, & Zhang, 2018), should be developed. Such a model, rather than only gene copies or methylation rates, may better reflect

in situ methylation potentials and MeHg production. Nevertheless, taxonomic identification and activity measurements of Hg methylators remain crucial components in studies of Hg contamination.

In terms of the methodology for species identification of Hg methylating microbes, current Hg methylation gene sequencing approaches include the traditional Sanger method with clone library construction by using primer sets targeting *Desulfobacterota*, *Methanomicrobia*, and *Firmicutes* (Bae, Dierberg, & Ogram, 2014; Liu et al., 2014; Schaefer, Kronberg, Morel, & Skyllberg, 2014), and later studies employed short-read (<300 bp) high-throughput sequencing (e.g., Illumina), and long-read (>1 kbp) high-throughput sequencing methods (e.g., PacBio, Nanopore) by using general NGS and group-specific primer sets (Christensen et al., 2016; Gionfriddo, Wymore, et al., 2020; Liu et al., 2018). Considering the low throughput and tedious process of the Sanger method even with the feature of long-read sequencing, most current studies have switched to using NGS sequencing approaches. The bias of the Illumina method is the short sequence length which only includes part of the *hgcAB* genes, while the two orders increase in error rates of long-read high throughput methods, relative to the short-read sequencing, is the bias inherent in the PacBio sequencing (Ardui, Ameur, Vermeesch, & Hestand, 2018; Gionfriddo, Wymore, et al., 2020). In order to explore the diversity of Hg methylating microbes, future studies need to further develop an updated reference database of *hgcAB* sequences which could be publicly accessed (Gionfriddo et al., 2019).

## 3. Demethylation

Demethylation (MeHg degradation) is the breakage of the C—Hg bond in MeHg and the subsequent transformations of the carbon and Hg moieties. Demethylation leads to a reduction in the concentration of MeHg that is available for bioaccumulation by organisms and food chains, alleviating the consequences of Hg contamination. While demethylation and its importance were discovered shortly after the discovery of Hg methylation (Furukawa, Suzuki, & Tonomura, 1969; Spangler, Spigarelli, Rose, & Miller, 1973), it has not been studied as extensively as methylation. Moreover, while current paradigms attribute methylation largely to the HgcAB-mediated microbial process (see above; Podar et al. (2015)), several biotic and abiotic processes are known for demethylation (Barkay & Gu, 2022). Understanding the dynamics of these processes and how they are controlled by environmental conditions is critical for mitigation of Hg

accumulation and toxicity and current lack of knowledge limits efforts to prevent and remediate Hg contamination.

Methylmercury may be degraded biotically and abiotically. In this review, we focus on biotic processes and the organisms that mediate them; abiotically, MeHg may be degraded by photochemical or dark processes. Briefly, in light-exposed environments, such as freshwater lakes, photodemethylation dominates (Sellers, Kelly, Rudd, & MacHutchon, 1996) and numerous studies examined this process mechanistically (summarized in Barkay and Gu (2021)). Photodemethylation accounts for up to 80% of MeHg degradation in light-exposed environments and is affected by the quantity and nature of dissolved organic matter (DOM) in natural waters. Degradation may be direct by photolysis of the C—Hg bond or indirect by the formation of reactive oxygen species (ROS) and free radicles. The seminal discovery that photodemthylation results in mass-independent fractionation (MIF) of Hg (Bergquist & Blum, 2007) has led to determinations of the role of photodemethylation in controlling MeHg bioavailability in numerous environments and in identifying sources of, and pathways leading to, MeHg accumulation in food chains (Tsui, Blum, & Kwon, 2020). Dark demethylation processes largely consist of processes that involve soluble or solid phase sulfides (West, Graham, Van, & Jonsson, 2020) and include the formation of dimethylmercury sulfide derivatives, $(CH_3Hg)_2S$, when monomethylmercury interacts with soluble and mineral-phase sulfides (Barkay & Gu, 2022). We note that because $H_2S$ is the final product of sulfate reduction, this process may be indirectly impacted by SRB as has been shown by Baldi, Pepi, and Filippelli (1993). Other abiotic demethylation processes are described in details by Barkay and Gu (2022).

Here, we are focusing on biotic demethylation, specifically describing demethylating microorganisms and the metabolic pathways and genes that they deploy in this process (Table 1). Readers with an interest in the topic are referred to several reviews of the role of demethylation in Hg biogeochemistry that have been published in recent years (Amin, Khan, Sarwar, Nawab, & Khan, 2021; Barkay & Gu, 2022; Du et al., 2019; Klapstein & O'Driscoll, 2018; Tsui et al., 2020).

## 3.1 Reductive and oxidative biotic demethylation processes

Biotic demethylation processes are distinguished based on the products of the degradation, Hg(II) vs Hg(0) and $CH_4$ vs $CO_2$ (Barkay & Gu, 2022). Reductive demethylation (RD) results in the formation of Hg(0) and

**Table 1** Biological demethylation: mechanisms and organisms.

| Mechanisms | Genes and proteins | Organisms | Comments | References |
|---|---|---|---|---|
| **Reductive demethylation (RD):** Organomercury Lyase (MerB); protonolytic cleavage of C—Hg in diverse organomercury compounds. Products include methane and Hg(0) | *merB* is part of broad-spectrum mercury-resistance (*mer*) operons. | Mostly heterotrophic aerobic bacteria; *Proteobacteria*, *Firmicutes*, *Actinobacteria* | • Hg(II), the product of MerB, is reduced by MerA to form Hg(0)<br>• The process is most common in oxic highly contaminated environments | Parks et al. (2009), Miller (2007), Pitts and Summers (2002), and Barkay and Gu (2022) |
| **Oxidative demethylation (OD):** Mechanism(s) likely co-metabolism of C1 utilization as an energy and carbon sources | Unknown | Sulfate reducers and methanogens | • Environmental incubations implicate OD in demethylation in many anoxic environments<br>• Lacking microbes that in culture perform OD, have limited molecular investigations of this process | Oremland, Culbertson, and Winfrey (1991), Marvin-Dipasquale and Oremland (1998), Baldi et al. (1993), and Barkay and Gu (2022) |
| **Methanotrophic demethylation (MD):** Activity is associated with methanobactin metabolism, its production and uptake | • Methanobactin is likely involved in the binding and transport of MeHg into the cell.<br>• Methanol dehydrogenase? | Methanotrophs:<br>• *Methylosinus trichosporium* OB3b<br>• *Methylocystis* sp. SB2 | • Activity is associated with central metabolism in methanotrophs<br>• Demethylation has only been observed with laboratory grown cultures | Lu et al. (2017) and Kang-Yun et al. (2022) |

$CH_4$ while oxidative demethylation (OD) produces Hg(II) and $CO_2$. This distinction is rooted in the history of demethylation research whereby degradation by resistant bacteria that degraded MeHg, likely by the *mer* system (see below), was the first to be discovered (Spangler, Spigarelli, Rose, Flippin, & Miller, 1973; Spangler, Spigarelli, Rose, & Miller, 1973). However, it was subsequently demonstrated that in environmental incubations to which radioactive MeHg, $^{14}CH_4Hg^+$, was added, degradation products included both $^{14}CO_2$ and $^{14}CH_4$ (Korthals & Winfrey, 1987; Oremland et al., 1991). The authors termed the unknown process of MeHg degradation to $CO_2$ and an unidentified Hg product, OD. Over time as more and more studies followed $^{14}$MeHg degradation by measuring the production of volatile $^{14}$C carbonaceous products (Ramlal, Rudd, & Hecky, 1986), the term RD became common to describe production of $CH_4$ from MeHg (Hines, Faganeli, Adatto, & Horvat, 2006).

Reductive demethylation is synonymous to *mer*-mediated demethylation where MeHg is degraded as part of the broadly distributed and ubiquitous microbial Hg resistance system (Barkay, Kritee, Boyd, & Geesey, 2010; Boyd & Barkay, 2012; Christakis, Barkay, & Boyd, 2021) to Hg(0) and $CH_4$. While the *mer* system is characterized mechanistically and genetically (Barkay, Miller, & Summers, 2003; Priyadarshanee, Chatterjee, Rath, Dash, & Das, 2022), much less is known about organisms, mechanisms, and genes that underpin OD. The later includes major groups of anaerobic microbes (Marvin-Dipasquale & Oremland, 1998) as well as the recently described demethylation by methanotrophic bacteria (Lu et al., 2017). Below, we briefly outline the current state of knowledge on biotic demethylation mechanisms (Table 1).

While we are using the accepted classification of RD vs OD, we note that the term RD is only accurate when the Hg(II) that results from the breakage of the C—Hg bond in MeHg is reduced to Hg(0), as in the case of *mer*-dependent demethylation. As $CH_4$ may be a partial product with $CO_2$ of demethylation processes that result in Hg(II) as a final product (Baldi et al., 1993; Marvin-Dipasquale & Oremland, 1998), relating to any process that results in $CH_4$ formation as RD is misleading.

## 3.2 Reductive demethylation, or *mer*-dependent degradation to Hg(0) and $CH_4$

The *mer*-dependent degradation of MeHg, and other organomercury compounds, is a function of the so-called broad-spectrum Hg resistance operons. Microorganisms carrying such operons are resistant to, and convert

organomercury compounds to Hg(0), by expressing *merB* which encodes for the organomercury lyase enzyme (MerB). MerB breaks the C—Hg bond in a broad range of organomercury compounds; the resulting Hg(II) is than reduced by the enzyme mercuric reductase (MerA) to Hg(0). As Hg(0) has high vapor pressure and low aqueous solubility, it is spontaneously removed from the immediate environment of the organism. Hence, the activities of broad-spectrum *mer* systems detoxify organomercury and facilitate microbial survival and activities in contaminated environments. Other functions encoded by *mer* operons include inorganic and organic Hg transporters, and regulatory functions that induce expression in presence of Hg and repress expression in its absence. *mer* operons and their individual functions are described below (see redox transformations of inorganic Hg), in recent publications (Christakis et al., 2021; Naguib, El-Gendy, & Khairalla, 2018; Priyadarshanee et al., 2022), and in older but useful ones (Barkay et al., 2003; Barkay & Wagner-Döbler, 2005).

### 3.2.1 Mechanism of MerB activity

The model system for the study of MerB is the enzyme encoded by the broad-spectrum Hg resistance plasmid R831 (Barkay & Gu, 2022; Begley, Walts, & Walsh, 1986b). The enzyme protonolytically cleaves the Hg—C bond in a broad range of organomercury compound by an $S_E2$ mechanism. Catalysis depends on the presence of two Cysteine (Cys) and an aspartic acid (Asp) residues in the enzyme's active site (Pitts & Summers, 2002) and the availability of a water molecule (Miller, 2007; Parks et al., 2009). Interaction with the substrate leads to changes in charge distribution among the thiolates of the Hg-bound Cys residues and the nearby Asp that weaken the C—Hg bond, exposing the bond to the protonolytic attack and the subsequent release of a reduced carbon moiety, $CH_4$ (when MeHg is the substrate), and Hg(II) (Melnick & Parkin, 2007; Miller, 2007; Parks et al., 2009). The resulting Hg(II) product likely remains bound to the two Cys residues in the active site, serving as a substrate to MerA (Benison et al., 2004). Thus the complete conversion of MeHg to Hg(0), a reductive process, depends on a direct interactions between MerB and MerA. For a detailed description of the mechanism of MerB, please see previous reports (Barkay & Gu, 2022；Lafrance-Vanasse, Lefebvre, Di Lello, Sygusch, & Omichinski, 2009；Miller, 2007; Parks et al., 2009).

### 3.2.2 Diversity, evolution, and distribution of MerB and microbes that reductively demethylate MeHg

Consistent with observations that RD largely occurs in aerated environments with high Hg concentrations (Barkay & Gu, 2022; Barkay & Wagner-Döbler, 2005), bacterial taxa that possess MerB and degrade MeHg are largely aerobic heterotrophs including pseudomonads (Clark, Weiss, & Silver, 1977), *Firmicutes* (Huang, Narita, Yamagata, & Endo, 1999; Matsui et al., 2016; Weiss, Murphy, & Silver, 1977), *Actinobacteria* (Ravel, DiRuggiero, Robb, & Hill, 2000), and enteric bacteria (Schottel, Mandal, Clark, Silver, & Hedges, 1974). *Geobacter bemidgiensis* Bam is the only obligate anaerobe in which MerB activity has been demonstrated (Lu et al., 2016). All these studies were performed with pure cultures grown in the laboratory. A broader picture on MerB carrying organisms is afforded in metagenomic databases. In a recent survey of 84,032 archaeal and bacterial genomes, metagenome assembled genomes, and single-cell genomes, Christakis et al. (2021) identified 1936 MerB homologs, 11 of which were identified in archaeal genomes and the remaining among bacterial genomes. To the best of our knowledge, MerB activity has not been reported in any archaeon. Most MerB homologs were found in genomes of the *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, but several were identified among taxa not previously known to be resistant to and degrade MeHg. Together, MerB-carrying genomes span broad taxonomic ranges that are commonly found in diverse environments and use diverse metabolisms for energy production and nutrient acquisition. The data suggest new niches where RD may occur. Identifying these niches and how MerB constrains MeHg accumulations in these niches would enhance remediation efforts in the diverse environments where Hg contamination occurs.

Functional and sequence diversities of MerB proteins are high. Functionally, the enzyme cleaves the C—Hg bond in substrates that vary in their chemical properties (Begley, Walts, & Walsh, 1986a) with different MerB exhibiting varied catalytic preferences and kinetics (Barkay & Gu, 2022; Chien et al., 2010). Moreover, MerB sequences are highly diverse sharing in some cases <30% sequence identity, and there is no relationship between the sequence diversity of MerBs and their substrate preference (Barkay & Gu, 2022). The practical consequence of this high diversity is that molecular tools to detect MerB in environmental metagenomes cannot be developed as these tools, e.g., PCR primers, depend on sequence similarity. The absence of such tools limits our ability to assess the role of *mer*-dependent RD in environmental incubations. To the best of our knowledge the only

study to show the involvement of MerB in demethylation by microbial communities was based on relating *merA* abundance to RD demethylation rates as measured by the production of $^{14}CH_4$ from environmental samples spiked with $^{14}$MeHg (Schaefer et al., 2004). This approach was justified as *merB* is often linked to *merA* in *mer* operons (see below and Barkay et al. (2003) and Barkay and Gu (2022)).

MerB has no amino acid sequence homology to any protein in databases. It has a partial structural homology to NosL, a Cu(I)-binding lipoprotein which is a part of the nitrous reductase system (Taubner, McGuirl, Dooley, & Copie, 2006); both enzymes share two "treble-clef"-like structures, typical to TRASH domains, in their active core. Having no sequence homology with other known proteins means that ancestry relationships cannot be determined, i.e., an evolutionary path for MerB cannot be deciphered. Moreover, it is impossible to understand what selective pressure drives the evolution of MerB as organomercury compounds that naturally occur in the environment, MeHg (Gilmour et al., 1998) and ethylmercury (Tomiyasu et al., 2017), are present at very low subtoxic concentrations. This observation together with evidence that MerB homologs are rarely found among early evolving microbial lineages led Boyd and Barkay (2012) to propose that MerB was recruited to the *mer* system once man-made organomercury reached the environment with industrial and agricultural contamination. To date, this proposition has not been tested experimentally.

In summary, RD, the *mer*-mediated process, is the best understood biological demethylation pathway. While the mechanistic details of this process are fairly well understood, its role and importance in Hg biogeochemistry are elusive. The broad diversity of MerB proteins complicates the development of molecular tools to interrogate RD in microbial communities that reside in environments where demethylation occurs.

## 3.3 Oxidative demethylation

Oxidative demethylation, a process that is defined by the generation of oxidized end-products, Hg(II) and $CO_2$ (Oremland et al., 1991), is the least understood biotic demethylation process (Barkay & Gu, 2022) (Table 1). Yet, OD commonly dominates demethylation in many environments such as peat soils (Marvin-DiPasquale et al., 2000), rice paddy soils (Wu et al., 2020), salt marsh and freshwater sediments (Oremland et al., 1991), riverine sediments (Oremland, Miller, Dowdle, Connell, & Barkay, 1995; Yu et al., 2012),

Hg mine runoffs (Gray & Hines, 2006; Marvin-DiPasquale, Agee, Bouse, & Jaffe, 2003), and coastal marine (Hines et al., 2000) and estuarine (Figueiredo et al., 2018) sediments. Intriguingly, OD often dominates in anoxic conditions as those under which methylation takes place. The reason we have such a limited understanding of OD is that to date we have no microorganisms in pure culture that perform OD; in absence of such pure cultures, biochemical, physiological, and genetic investigations are limited.

Examining $^{14}$MeHg degradation in environmental slurries incubated in presence of the specific metabolic inhibitors, 2-bromoethanesulfonicacid (BES) inhibiting methanogenesis, and molybdate ($MoO_4^{=}$), an inhibitor of sulfate reduction, and the stimulation of OD by the addition of sulfate to incubations, implicated both methanogens and SRB in OD (Marvin-Dipasquale & Oremland, 1998). Based on these observations the authors suggested that OD was co-metabolically related to C1 metabolism, e.g., monomethylamine degradation by methanogens:

$$4CH_3Hg^+ + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4Hg^{2+} + 4H_2$$

A process that would produce a 3:1 M ratio of $CH_4$:$CO_2$, or e.g., acetate oxidation by SRB:

$$SO_4^{2-} + CH_3Hg^+ + 3H^+ \rightarrow H_2S + CO_2 + Hg^{2+} + 2H_2O$$

a process that would exclusively produce $CO_2$. However, when pure cultures of methanogens and SRB were tested for demethylation, the incubations degraded a minor fraction of the added $^{14}CH_3Hg^+$ and the addition of C1 substrates had no significant effects on the demethylation rates or the identity of their C1 gaseous products (Oremland et al., 1991). Because many methylating SRB also degrade MeHg (Bridou, Monperrus, Gonzalez, Guyoneaud, & Amouroux, 2011; Gilmour et al., 2011; Graham, Bullock, Maizel, Elias, & Gilmour, 2012), it is possible that OD could occur by a reversed Hg methylation reaction. This hypothesis was tested by Pak and Bartha (1998b) who showed exclusive production of $^{14}CH_4$ when pure cultures of strong methylators, e.g., strain ND132$^T$, were incubated with $^{14}CH_3Hg^+$. It should however be noted that the authors did not report a full recovery of the added $^{14}$MeHg in their incubations.

While many studies have implicated SRB in OD, this process may be only indirectly mediated by organisms that produce $H_2S$. It has been known for decades that monoMeHg in the presence of $H_2S$ would chemically produce diMeHg and HgS (Deacon, 1978; Rowland, Davies, & Grasso, 1977),

a process that was proposed by Baldi et al. (1993) to explain demethylation by SRB. The authors showed a spontaneous precipitation of $CH_3Hg^+$ as $(CH_3Hg)_2S$ with biogenic $H_2S$ and the conversion of $(CH_3Hg)_2S$, to diMeHg and metacinnabar (HgS). diMeHg was further converted to $CH_4$ and $CH_3Hg^+$. Overall, two molecules of monoMeHg were converted to HgS, one monoMeHg, and $CH_4$. Aqueous- and solid-phase sulfides (Kanzler et al., 2018; West et al., 2020) have been implicated in dark abiotic demethylation (Barkay & Gu, 2022). In view of the biogenic origin of sulfides in many environments, these processes may be considered indirect biotic demethylation.

In summary, although OD is the dominant mechanism of MeHg degradation in anoxic environments where Hg methylation occurs, little is known about its mechanism, thus limiting our ability to use this process in remediation. This lack of knowledge is likely due to the absence of specific organisms that carry out OD in laboratory cultures. Traditionally, the role of specific mechanisms in biogeochemical processes is first deciphered in microbes that carry out the process as pure cultures and then interrogating the processes in environmental incubations and samples collected in the field. In recent decades, metagenomic tools which are largely based on knowledge of genes and enzymatic processes, have had a major role in such investigations (Grossart, Massana, McMahon, & Walsh, 2020; Madsen, 2011). In the case of OD, the process was discovered in environmental incubations (Korthals & Winfrey, 1987; Oremland et al., 1991) and the isolation of cultures that oxidatively degrade MeHg remains a challenge more than three decades later. Additionally, OD may be a function that requires interactions among several microbes. If so, the enrichment of consortia of oxidative demethylators from environmental incubations might be useful, together with recent guild-based approaches to metagenome analysis. In the later, the dynamics of species co-abundance in environmental metagenomes under changing conditions suggests cooperative functionalities (Wu, Zhao, Zhang, Lam, & Zhao, 2021).

## 3.4 Methanotrophic demethylation

The most recently discovered biotic demethylation mechanism is mediated by methanotrophic bacteria. This process seems to be associated with the central metabolism of methanotrophes (Kang-Yun et al., 2022; Lu et al., 2017; Vorobev et al., 2013), hence the term methanotrophic demethylation (MD) (Table 1). Demethylation depends on the production (Lu et al., 2017;

Vorobev et al., 2013) or presence (Kang-Yun et al., 2022) of methanobactin, a chelator produced by methanotrophs to assure supply of copper to the particulate methane monooxygenase. This enzyme converts $CH_4$ to methanol initiating the utilization of $CH_4$ as an energy and growth substrate (Strong, Xie, & Clarke, 2015). However, methanobactin is not sufficient for demethylation and because MeHg degradation was inhibited by the addition of methanol (Baesman et al., 2015; Lu et al., 2017), the authors hypothesized that the enzyme methanol dehydrogenase breaks the C—Hg bond in MeHg in analogy to its action on the C—H bond in methanol (Lu et al., 2017). To date, testing of this hypothesis has not been reported (Barkay & Gu, 2022). Unlike *mer*-mediated demethylation, MD takes place at very low, environmentally relevant concentrations, pM to nM MeHg, producing Hg(II) as its final product (Lu et al., 2017).

The idea that methanotrophs and methylotrophs are engaged in demethylation has been considered early on at the time when OD was discovered (Oremland et al., 1991). This pathway has been deemed unlikely because demethylation was tested by following the gaseous carbon products, $^{14}CH_4$ and $^{14}CO_2$, and the assumption that in methanogenic environments the large amount of $CH_4$ and other C-1 substrates would lead to radioisotope dilution. This consideration is still valid because to date MD has only been shown in laboratory cultures. Thus, demonstrating MD in environmental incubations is essential to integrating this activity into our paradigms of the geochemical cycling of Hg. One environment where MD may play a critical role in modulating MeHg accumulation is polar and subpolar regions where climate-induced permafrost thawing has resulted in significant increases of MeHg concentrations in pore water and run off (Gordon, Quinton, Branfireun, & Olefeldt, 2016; Schuur & Mack, 2018) and its formation (Roth et al., 2021; Tarbier, Hugelius, Sannel, Baptista-Salazar, & Jonsson, 2021). Permafrost-underlined northern wetlands are dominated by *Sphagnum* moss (Shirokova et al., 2021; Vitt, Halsey, & Zoltai, 1994) and methanotrophic bacteria live as intracellular symbionts in *Sphagnum* moss (Kip et al., 2011, 2010) where they control emissions of $CH_4$ to the atmosphere (Larmola et al., 2010).

Other biotic demethylation processes may occur (Barkay & Gu, 2022) and will likely be studied in the future. Most particularly, the possibility of assimilative demethylation whereby the carbon moiety would be partially integrated in the demethylating biomass as reported for an acetogen (Oremland et al., 1991) and as expected for MD, should be explored.

## 4. Redox transformations of inorganic mercury

Inorganic Hg may exist in three oxidation states, Hg(0), Hg(I), and Hg(II). Because Hg(I) readily disproportions to Hg(0) and Hg(II) (Latimer, 1952), Hg(II) and Hg(0) are the dominating oxidation states of inorganic Hg in the environment. Within the paradigm of the Hg biogeochemical cycle (Fig. 1) and our focus on MeHg, the oxidation states of inorganic Hg affect bioavailability to methylating microbes. With Hg(II) serving as a substrate for methylation and Hg(0) partitioning to the atmosphere due to its low aqueous solubility and high vapor pressure (Toribara, Shields, & Koval, 1970), any transformation that increases the amount of Hg as Hg(II) has the potential to stimulate methylation, and conversely, reactions that reduce Hg(II) to Hg(0) may constrain methylation. Together these reactions constitute the "Hg redox wheel" (Branfireun, Cosio, Poulain, Riise, & Bravo, 2020; Grégoire & Poulain, 2018). In light-exposed environments, the photoreduction of Hg(II) to Hg(0) is a dominant process (Costa & Liss, 2000; Luo, Cheng, & Pan, 2020; Nriagu, 1994). However, in dark environments as often occurs in anoxic sediments and bottom waters where methylation takes place (Poulain et al., 2004; Rolfhus & Fitzgerald, 2004), a variety of processes reduce Hg(II) to Hg(0) limiting production of MeHg. These processes, specifically those mediated by microorganisms, are described below.

### 4.1 Inorganic mercury reduction

The Hg resistance (*mer*)-mediated system is by far the best understood Hg(II) reduction process whereby a dedicated system detoxifies Hg(II), and sometimes organomercury compounds (see section on demethylation above), by its conversion to volatile Hg(0) (Barkay et al., 2003; Barkay & Wagner-Döbler, 2005). The central function of the *mer* system is the enzyme mercuric reductase (MerA), a pyridine-nucleotide disulfide oxidoreductase (Fox & Walsh, 1982), which uses electrons originating in NAD(P)H to intracellularly convert Hg(II) to Hg(0). Elemental Hg then diffuses out of the cell and may be partitioned into the gaseous phase due to its high volatility, thus removing Hg from the immediate environment of the organism (Barkay et al., 2003). In addition to MerA, the *mer* system includes Hg(II) transporters as well as regulatory genes (Summers, 1986) that assure expression of the operon only in presence of Hg (Summers, 1992).

*mer* is common among both Bacteria and Archaea that possess diverse metabolisms and are present in diverse environments (Barkay et al., 2010; Christakis et al., 2021). However, with few exceptions (Lu et al., 2016), *mer* is largely found among obligate and facultative aerobes leading to the hypothesis that its distribution is constrained by the redox state of the environment (Barkay et al., 2010). The rationale supporting this hypothesis is that it is only in oxygen-replete environments that Hg exists as Hg(II), a highly toxic form that necessitates efficient and rapid detoxification (Barkay et al., 2010).

*mer*-mediated reduction likely plays an important role in the removal of Hg(0) to the atmosphere in contaminated environments. It has been suggested, and regrettably accepted within the Hg biogeochemical cycle paradigm, that the dependency of *mer* gene expression on Hg(II) renders it ineffective in most environments where Hg concentrations are typically at the pM to nM range (Morel, Kraepiel, & Amyot, 1998). Nevertheless, *mer* expression at low, environmentally-relevant Hg concentrations has been documented (Kelly, Rudd, & Holoka, 2003; Ralston & O'Halloran, 1990) and *merA* transcripts were detected in an uncontaminated (90 pM total Hg) riverine microbial biomass (Nazaret, Jeffrey, Saouter, Von Haven, & Barkay, 1994).

The understanding of how the *mer* system works has resulted in numerous applications in environmental remediation (Kumari, Amit, Jamwal, Mishra, & Singh, 2020; Velasquez-Riano & Benavides-Otaya, 2016) including the removal of Hg from industrial wastes using bioreactors (Wagner-Dobler, 2003), the construction of transgenic plants (Liu et al., 2020), the use of biosorbents (Kostal, Mulchandani, Gropp, & Chen, 2003), and the construction and deployment of Hg biosensors (Bose, Maity, & Sarkar, 2021).

It has been known for a long time that other mechanisms for biotic Hg(II) reduction exist, in addition to the *mer* operon (Alberts, Schindler, Miller, & Nutter, 1974; Allard & Arsenie, 1991). Evidence supporting this proposition included the formation of dissolved gaseous Hg (DGM) pools, largely in oxygen-limited zones within water bodies, under conditions that are likely to exclude *mer* involvement (Lamborg et al., 2021; Poulain et al., 2004). More recently, several mechanisms for *mer*-independent biotic reduction have been discovered.

1. Reduction of Hg(II) during ferrous iron oxidation by chemoautotrophic bacteria—Some acidophilic bacilli, e.g., *Thiobacillus ferrooxidans*, reduce Hg(II) to Hg(0) in a Fe(II)-dependent process. This activity was

enhanced by the addition of cytochrome C oxidase and rusticyanin and was inhibited by cyanide. These results led the authors to suggest that Hg(II) was reduced at the end of a respiratory electron chain where Hg(II) replaced oxygen as a terminal electron acceptor (Iwahori, Takeuchi, Kamimura, & Sugio, 2000; Sugio et al., 2001; Takeuchi et al., 2001). While active strains were 10–100 times more sensitive to Hg than *Thiobacilli* with an active *mer* system, the authors identified strains that possessed Hg-resistant oxidases and used these strains to remove 94% of Hg from contaminated Fe(II)-amended soils in 30 days of incubation (Takeuchi, Iwahori, Kamimura, & Sugio, 1999).

2. Reduction of Hg(II) by Hg-sensitive ferric iron reducing bacteria—The Hg methylating iron reducing bacterium *Geobacter* spp. and the non-methylator *Shewanelle onidensis* MR1, convert Hg(II) to Hg(0), an activity that was enhanced by preincubation under Fe(III) reducing conditions prior to the addition of Hg(II) to growing cultures (Wiatrowski, Ward, & Barkay, 2006). In *G. sulfurreducens,* Hg(II) reduction depends on sorption of Hg(II) to cell surfaces and its rate declined significantly in mutants lacking several cytochromes (Hu et al., 2013). These observations suggested a coupling between Fe(III) and Hg(II) reductions that could possibly be explained by the demonstration of a rapid kinetic reduction of Hg(II) by magnetite (Wiatrowski et al., 2009). Mössbauer spectroscopic analysis revealed a decrease in Fe(II) content in the mineral lettice, corresponding to the oxidation of Fe(II) to Fe(III) in magnetite when Hg(II) was reduced (Wiatrowski et al., 2009). Together, the data suggest a reduction of Hg(II) to Hg(0) by Fe(II), the product of Fe(III) reduction. Thus, this reaction is an abiotic reduction that may be indirectly mediated by microbial metabolites. This explanation is consistent with Hg(II) reduction by Fe(II)-containing minerals (Amirbahman, Kent, Curtis, & Marvin-DiPasquale, 2013; Etique et al., 2021; Wang et al., 2021) and offers a link between Hg biogeochemistry and the iron content and reduction rates in contaminated environments (Harris-Hellal, Grimaldi, Garnier-Zarli, & Bousserrhine, 2011; Warner et al., 2003). This mode of Hg(II) reduction, occurring, as is Hg(II) methylation, under anoxic conditions, may significantly constrain MeHg production.
3. Reduction of Hg(II) by photoheterotrophs—A role for phototrophic bacteria in Hg(II) reduction was suggested by studies in stratified lakes where Hg(0) concentrations peaked in the metalimnion (Poulain et al., 2004), an ecological niche favored by anoxygenic sulfur and

non-sulfur phototrophic bacteria. Grégoire and Poulain (2016) used the model purple-non-sulfur bacteria *Rhodobacter capsulatus*, *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* to show that these organisms reduced Hg(II) to Hg(0) in a light-depended mode when provided with reduced carbon source. Growth under such myxotrophic conditions is challenging due to the production of excessive reducing power and the resulting redox imbalance when cells are starved for oxidized electron acceptors (Green & Paget, 2004). A connection between Hg(II) reduction and redox imbalance was established by showing significant growth enhancement when cultures were grown in presence of 200 nM Hg(II) relative to no Hg controls in medium that favored accumulation of NADH relative to $NAD^+$ (Grégoire & Poulain, 2016).

4. Reduction of Hg(II) by chemotrophic fermenting microorganisms—The discovery of photoheterotrophic Hg(II) reduction (see above) had raised questions about its possible role in controlling MeHg production in rice paddies. Rice paddies are a major source for MeHg accumulation in rice posing public health risks to millions of consumers (Rothenberg, Windham-Myers, & Creswell, 2014). Rice paddies are a niche where heliobacteria, anoxygenic phototrophs that can also grow by fermentation, are common (Asao & Madigan, 2010). Indeed, the model *Heliobacterium modesticaldum* reduced Hg(II) when grown both phototrophically and by fermentation of pyruvate (Gregoire, Lavoie, & Poulain, 2018). The authors subsequently showed that other anaerobes such as *Clostridium acetobutylicum*, an obligate fermenter, and the iron reducer *G. sulfurreducens*, reduced Hg(II) and this activity depended on the enzyme pyruvate:ferredoxin oxidoreductase. Thus, a reduced ferredoxin is likely an electron donor in Hg(II) reduction (Gregoire et al., 2018). Considering that fermentation is a major microbial pathway for the conservation of energy in anoxic environments and that fermenting microbes occupy the same environmental niche as Hg methylators, chemotrophic fermentative reduction of Hg(II) may significantly constrain MeHg production.

In all of these mechanisms, Hg(II) serves as a sink for electrons that are produced during microbial metabolism: Fe(II) oxidation and Fe(III) reduction (mechanisms 1 and 2, respectively), and the oxidation of carbon sources (mechanisms 3 and 4). Here, the electrophilic properties of Hg(II), the most oxidized form of this element, likely promote these activities. While rates of reduction may be slower than rates observed with organisms that carry the dedicated *mer*-system (Gregoire et al., 2018; Wiatrowski et al., 2006), the

abundance of active iron reducers and fermenters in anoxic environments is many orders of magnitude higher than that of *mer*-carrying microbes. Thus, in terms of competing with methylation for the same substrate, Hg(II), these newly discovered mechanisms may be useful in mitigation strategies of Hg-contaminated environments.

## 4.2 Inorganic Hg oxidation

The oxidation of Hg(0) to Hg(II) is by far the one juncture in the Hg biogeochemical cycle of which we know the least. The recent discovery of uptake of Hg(0) in Arctic tundra ecosystems (Obrist et al., 2017) brings the importance of this transformation to the fore. Chemical oxidation of Hg(0) is well known and is broadly used to remove Hg from flue gases to prevent contamination resulting from power generation (Gao et al., 2013; Liu, Liu, & Wang, 2021; Xing et al., 2017). Aerobic microorganisms, e.g., *Escherichia coli* may oxidize Hg(0) to Hg(II) in a process that depends on the activity of KatG, a catalase, and it is thus related to oxidative stress response among aerobes (Smith, Pitts, McGarvey, & Summers, 1998). More recently, Hg(0) oxidation to Hg(II) and methylation were described in anaerobes that may or may not methylate Hg, such as SRB (Colombo et al., 2013; Colombo, Ha, Reinfelder, Barkay, & Yee, 2014) and FeRB (Hu, Lin, Zheng, Tomanicek, et al., 2013). Many of these anaerobes lack oxidative stress response. Little is known about how anaerobes oxidize Hg(0). Wang, Schaefer, Mishra, and Yee (2016) showed that in *P. Mercurii* ND132$^{T}$ oxidation occurred mostly intracellularly and depended on the cytoplasm thiol content. The latest development in biotic Hg(0) oxidation was the description of a microbial consortium (Huang et al., 2020) that coupled Hg(0) oxidation to nitric oxide reduction by processes depending on sulfur oxidation and disproportionation of thiosulfate to sulfide and sulfate. The oxidation resulted in precipitation of Hg as ß-HgS. Activities were demonstrated in a simulated flue gases mixture, suggesting that this consortium may serve as the basis of decontamination of industrial emissions (Huang et al., 2020). As emissions of Hg(0) during power generation remains a major source of environmental Hg contamination (Balasundaram & Sharma, 2019; Raj & Maiti, 2019), more knowledge on how microbes oxidize Hg(0) to Hg(II) that may then be methylated should be pursued.

## Acknowledgments

This research was supported by the National Science Foundation 2117703 award (to R.Y.).

## References

Aaseth, J., Wallace, D. R., Vejrup, K., & Alexander, J. (2020). Methylmercury and developmental neurotoxicity: A global concern. *Current Opinion in Toxicology*, *19*, 80–87.

Ahmed, M., & Lin, L. S. (2017). Ferric reduction in organic matter oxidation and its applicability for anaerobic wastewater treatment: A review and future aspects. *Reviews in Environmental Science and Biotechnology*, *16*, 273–287.

Alberts, J. J., Schindler, J. E., Miller, R. W., & Nutter, D. E. (1974). Elemental mercury evolution mediated by humic acid. *Science*, *184*, 895–896.

Allard, B., & Arsenie, I. (1991). Abiotic reduction of mercury by humic substances in aquatic system - an important process for the mercury cycle. *Water, Air, and Soil Pollution*, *56*, 457–464.

Amin, S., Khan, S., Sarwar, T., Nawab, J., & Khan, M. A. (2021). Mercury methylation and its accumulation in rice and paddy soil in degraded lands: A critical review. *Environmental Technology and Innovation*, *23*.

Amirbahman, A., Kent, D. B., Curtis, G. P., & Marvin-DiPasquale, M. C. (2013). Kinetics of homogeneous and surface-catalyzed mercury(II) reduction by iron(II). *Environmental Science & Technology*, *47*, 7204–7213.

Ardui, S., Ameur, A., Vermeesch, J. R., & Hestand, M. S. (2018). Single molecule real-time (SMRT) sequencing comes of age: Applications and utilities for medical diagnostics. *Nucleic Acids Research*, *46*, 2159–2168.

Asao, M., & Madigan, M. T. (2010). Taxonomy, phylogeny, and ecology of the heliobacteria. *Photosynthesis Research*, *104*, 103–111.

Austin, D., Scharf, R., Carroll, J., & Enochs, M. (2016). Suppression of hypolimnetic methylmercury accumulation by liquid calcium nitrate amendment: Redox dynamics and fate of nitrate. *Lake and Reservoir Management*, *32*, 61–73.

Badalamenti, J. P., Summers, Z. M., Chan, C. H., Gralnick, J. A., & Bond, D. R. (2016). Isolation and genomic characterization of '*Desulfuromonas soudanensis* WTL', a metal- and electrode-respiring bacterium from anoxic deep subsurface brine. *Frontiers in Microbiology*, *7*, 913.

Bae, H. S., Dierberg, F. E., & Ogram, A. (2014). Syntrophs dominate sequences associated with the mercury methylation-related gene *hgcA* in the water conservation areas of the Florida Everglades. *Applied and Environmental Microbiology*, *80*, 6517–6526.

Bae, H. S., Dierberg, F. E., & Ogram, A. (2019). Periphyton and flocculent materials are important ecological compartments supporting abundant and diverse mercury methylator assemblages in the Florida Everglades. *Applied and Environmental Microbiology*, *85*(13), e00156–19. https://doi.org/10.1128/AEM.00156-19.

Baesman, S. M., Miller, L. G., Wei, J. H., Cho, Y., Matys, E. D., Summons, R. E., et al. (2015). Methane oxidation and molecular characterization of methanotrophs from a former mercury mine impoundment. *Microorganisms*, *3*, 290–309.

Bailey, L. T., Mitchell, C. P. J., Engstrom, D. R., Berndt, M. E., Wasik, J. K. C., & Johnson, N. W. (2017). Influence of porewater sulfide on methylmercury production and partitioning in sulfate-im-pacted lake sediments. *Science of the Total Environment*, *580*, 1197–1204.

Baker, B. J., Lazar, C. S., Teske, A. P., & Dick, G. J. (2015). Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria. *Microbiome*, *3*, 14. https://doi.org/10.1186/s40168-015-0077-6.

Balasundaram, K., & Sharma, M. (2019). Technology for mercury removal from flue gas of coal based thermal power plants: A comprehensive review. *Critical Reviews in Environmental Science and Technology*, *49*, 1700–1736.

Baldi, F., Pepi, M., & Filippelli, M. (1993). Methylmercury resistance in *Desulfovibrio desulfuricans* strains in relation to methylmercury degradation. *Applied and Environmental Microbiology*, *59*, 2479–2485.

Barkay, T., & Gu, B. (2022). Demethylation—the other side of the mercury methylation coin: A critical review. *ACS Environmental Au*, *2*, 77–97.

Barkay, T., Kritee, K., Boyd, E., & Geesey, G. (2010). A thermophilic bacterial origin and subsequent constraints by redox, light and salinity on the evolution of the microbial mercuric reductase. *Environmental Microbiology*, *12*, 2904–2917.

Barkay, T., Miller, S. M., & Summers, A. O. (2003). Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiology Reviews*, *27*, 355–384.

Barkay, T., & Wagner-Döbler, I. (2005). Microbial transformations of mercury: Potentials, challenges, and achievements in controlling mercury toxicity in the environment. *Advances in Applied Microbiology*, *57*, 1–52.

Begley, T. P., Walts, A. E., & Walsh, C. T. (1986a). Bacterial organomercurial lyase: Overproduction, isolation, and characterization. *Biochemistry*, *25*, 7186–7192.

Begley, T. P., Walts, A. E., & Walsh, C. T. (1986b). Mechanistic studies of a protonolytic organomercurial cleaving enzyme - bacterial organomercurial lyase. *Biochemistry*, *25*, 7192–7200.

Benison, G. C., Di Lello, P., Shokes, J. E., Cosper, N. J., Scott, R. A., Legault, P., et al. (2004). A stable mercury-containing complex of the organomercurial lyase MerB: Catalysis, product release, and direct transfer to MerA. *Biochemistry*, *43*, 8333–8345.

Benoit, J. M., Gilmour, C. C., Heyes, A., Mason, R. P., & Miller, C. L. (2003). Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In Y. Cai, & O. C. Braids (Eds.), *Biogeochemistry of environmentally important trace elements* (pp. 262–297). Washington, D. C: American Chemical Society Symposium Series.

Benoit, J. M., Gilmour, C. C., Mason, R. P., & Heyes, A. (1999). Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediments pore waters. *Environmental Science & Technology*, *33*, 951–957.

Bergquist, B. A., & Blum, J. D. (2007). Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems. *Science*, *318*, 417–420.

Berman, M., & Bartha, R. (1986). Levels of chemical versus biological methylation of mercury in sediments. *Bulletin of Environmental Contamination and Toxicology*, *36*, 401–404.

Berman, M., Chase, T., & Bartha, R. (1990). Carbon flow in mercury biomethylation by *Desulfovibrio desulfuricans*. *Applied and Environmental Microbiology*, *56*, 298–300.

Beutel, M. W., Duvil, R., Cubas, F. J., Matthews, D. A., Wilhelm, F. M., Grizzard, T. J., et al. (2016). A review of managed nitrate addition to enhance surface water quality. *Critical Reviews in Environmental Science and Technology*, *46*, 673–700.

Bone, S. E., Bargar, J. R., & Sposito, G. (2014). Mackinawite (FeS) reduces mercury(II) under sulfidic conditions. *Environmental Science & Technology*, *48*, 10681–10689.

Boone, D. R. (1987). Request for an opinion: Replacement of the type strain of *Methanobacterium formicicum* and reinstatement of *Methanobacterium bryantii* sp. nov. nom. Rev. (ex Balch and Wolfe, 1981) with M.o.H. (DSM 863) as the type strain. *International Journal of Systematic Bacteriology*, *37*, 172–173.

Boone, D. R., & Bryant, M. P. (1980). Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. *Applied and Environmental Microbiology*, *40*, 626–632.

Bose, S., Maity, S., & Sarkar, A. (2021). Review of microbial biosensor for the detection of mercury in water. *Environmental Quality Management*, 1–12.

Boyd, E. S., & Barkay, T. (2012). The mercury resistance operon: From an origin in a geothermal environment to an efficient detoxification machine. *Frontiers in Microbiology*, *3*, 349. https://doi.org/10.3389/fmicb.2012.00349.

Branfireun, B. A., Cosio, C., Poulain, A. J., Riise, G., & Bravo, A. G. (2020). Mercury cycling in freshwater systems - An updated conceptual model. *Science of the Total Environment*, *745*, 140906.

Bravo, A. G., Bouchet, S., Tolu, J., Bjorn, E., Mateos-Rivera, A., & Bertilsson, S. (2017). Molecular composition of organic matter controls methylmercury formation in boreal lakes. *Nature Communications*, *8*, 14255. https://doi.org/10.1038/ncomms14255.

Bravo, A. G., & Cosio, C. (2020). Biotic formation of methylmercury: A bio-physico-chemical conundrum. *Limnology and Oceanography*, *65*, 1010–1027.

Bravo, A. G., Peura, S., Buck, M., Ahmed, O., Mateos-Rivera, A., Ortega, S. H., et al. (2018). Methanogens and iron-reducing bacteria: The overlooked members of mercury-methylating microbial communities in Boreal Lakes. *Applied and Environmental Microbiology*, *84*, e01774-01718.

Bravo, A. G., Zopfi, J., Buck, M., Xu, J. Y., Bertilsson, S., Schaefer, J. K., et al. (2018). *Geobacteraceae* are important members of mercury-methylating microbial communities of sediments impacted by waste water releases. *The ISME Journal*, *12*, 802–812.

Bridou, R., Monperrus, M., Gonzalez, P. R., Guyoneaud, R., & Amouroux, D. (2011). Simultaneous determination of mercury methylation and demethylation capacities of various sulfate-reducing bacteria using species-specific isotopic tracers. *Environmental Toxicology and Chemistry*, *30*, 337–344.

Bryant, M. P., Wolin, E. A., Wolin, M. J., & Wolfe, R. S. (1967). *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. *Archiv für Mikrobiologie*, *59*, 20–31.

Cao, J. W., Gayet, N., Zeng, X., Shao, Z. Z., Jebbar, M., & Alain, K. (2016). *Pseudodesulfovibrio indicus* gen. Nov., sp nov., a piezophilic sulfate-reducing bacterium from the Indian Ocean and reclassification of four species of the genus *Desulfovibrio*. *International Journal of Systematic and Evolutionary Microbiology*, *66*, 3904–3911.

Carrell, A. A., Schwartz, G. E., Cregger, M. A., Gionfriddo, C. M., Elias, D. A., Wilpiszeski, R. L., et al. (2021). Nutrient exposure alters microbial composition, structure, and mercury methylating activity in periphyton in a contaminated watershed. *Frontiers in Microbiology*, *12*, 647861. https://doi.org/10.3389/fmicb.2021.647861.

Chen, Y., Bonzongo, J. C. J., Lyons, W. B., & Miller, G. C. (1997). Inhibition of mercury methylation in anoxic freshwater sediment by group VI anions. *Environmental Toxicology and Chemistry*, *16*, 1568–1574.

Chételat, J., Ackerman, J. T., Eagles-Smith, C. A., & Hebert, C. E. (2020). Methylmercury exposure in wildlife: A review of the ecological and physiological processes affecting contaminant concentrations and their interpretation. *Science of the Total Environment*, *711*, 135117.

Chiasson-Gould, S. A., Blais, J. M., & Poulain, A. J. (2014). Dissolved organic matter kinetically controls mercury bioavailability to bacteria. *Environmental Science & Technology*, *48*, 3153–3161.

Chien, M. F., Narita, M., Lin, K. H., Matsui, K., Huang, C. C., & Endo, G. (2010). Organomercurials removal by heterogeneous *merB* genes harboring bacterial strains. *Journal of Bioscience and Bioengineering*, *110*, 94–98.

Choi, S. C., & Bartha, R. (1993). Cobalamin-mediated mercury methylation by *Desulfovibrio desulfuricans* LS. *Applied and Environmental Microbiology*, *59*, 290–295.

Choi, S. C., Chase, T., & Bartha, R. (1994). Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. *Applied and Environmental Microbiology*, *60*, 4072–4077.

Choi, S. C., Chase, T., Jr., & Bartha, R. (1994). Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. *Applied and Environmental Microbiology*, *60*, 1342–1346.

Christakis, C. A., Barkay, T., & Boyd, E. S. (2021). Expanded diversity and phylogeny of *mer* genes broadens mercury resistance paradigms and reveals an origin for MerA among thermophilic archaea. *Frontiers in Microbiology*, *12*, 682605. https://doi.org/10.3389/fmicb.2021.682605.

Christensen, G. A., Gionfriddo, C. M., King, A. J., Moberly, J. G., Miller, C. L., Somenahally, A. C., et al. (2019). Determining the reliability of measuring mercury cycling gene abundance with correlations with mercury and methylmercury concentrations. *Environmental Science & Technology*, *53*, 8649–8663.

Christensen, G. A., Somenahally, A. C., Moberly, J. G., Miller, C. M., King, A. J., Gilmour, C. C., et al. (2018). Carbon amendments alter microbial community structure and net mercury methylation potential in sediments. *Applied and Environmental Microbiology*, *84*, (e01049-01017).

Christensen, G. A., Wymore, A. M., King, A. J., Podar, M., Hurt, R. A., Jr., Santillan, E. U., et al. (2016). Development and validation of broad-range qualitative and clade-specific quantitative molecular probes for assessing mercury methylation in the environment. *Applied and Environmental Microbiology*, *82*, 6068–6078.

Clark, D. L., Weiss, A. A., & Silver, S. (1977). Mercury and organomercurial resistances determined by plasmids in *Pseudomonas*. *Journal of Bacteriology*, *132*, 186–196.

Cohan, F. M. (2002). What are bacterial species? *Annual Review of Microbiology*, *56*, 457–487.

Colin, A., Schmidt, C., Pokrovski, G. S., Wilke, M., Borisova, A. Y., & Toplis, M. J. (2020). In situ determination of sulfur speciation and partitioning in aqueous fluid-silicate melt systems. *Geochemical Perspectives Letters*, *14*, 31–35.

Colombo, M. J., Ha, J. Y., Reinfelder, J. R., Barkay, T., & Yee, N. (2013). Anaerobic oxidation of Hg(0) and methylmercury formation by *Desulfovibrio desulfuricans* ND132. *Geochimica et Cosmochimica Acta*, *112*, 166–177.

Colombo, M. J., Ha, J., Reinfelder, J. R., Barkay, T., & Yee, N. (2014). Oxidation of Hg(0) to Hg(II) by diverse anaerobic bacteria. *Chemical Geology*, *363*, 334–340.

Compeau, G. C., & Bartha, R. (1985). Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. *Applied and Environmental Microbiology*, *50*, 498–502.

Cooper, C. J., Zheng, K., Rush, K. W., Johs, A., Sanders, B. C., Pavlopoulos, G. A., et al. (2020). Structure determination of the HgcAB complex using metagenome sequence data: Insights into microbial mercury methylation. *Communications Biology*, *3*, 320.

Cord-Ruwisch, R., Lovley, D. R., & Schink, B. (1998). Growth of *Geobacter sulfurreducens* with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. *Applied and Environmental Microbiology*, *64*, 2232–2236.

Cornell, R. M., & Schwertmann, U. (2003). *The iron oxides: Structure, properties, reactions, occurences, and uses*. New York: Wiley-VCH.

Correia, R. R. S., & Guimaraes, J. R. D. (2017). Mercury methylation and sulfate reduction rates in mangrove sediments, Rio de Janeiro, Brazil: The role of different microorganism consortia. *Chemosphere*, *167*, 438–443.

Costa, M., & Liss, P. (2000). Photoreduction and evolution of mercury from seawater. *Science of the Total Environment*, *261*, 125–135.

Dalcin Martins, P., Danczak, R. E., Roux, S., Frank, J., Borton, M. A., Wolfe, R. A., et al. (2018). Viral and metabolic controls on high rates of microbial sulfur and carbon cycling in wetland ecosystems. *Microbiome*, *6*, 138.

Date, S. S., Parks, J. M., Rush, K. W., Wall, J. D., Ragsdale, S. W., & Johs, A. (2019). Kinetics of enzymatic mercury methylation at nanomolar concentrations catalyzed by HgcAB. *Applied and Environmental Microbiology*, *85*, e00438–19. https://doi.org/10.1128/AEM.00438-19.

de Chaves, M. G., Silva, G. G. Z., Rossetto, R., Edwards, R. A., Tsai, S. M., & Navarrete, A. A. (2019). *Acidobacteria* ssubgroups and their metabolic potential for carbon degradation in sugarcane soil amended with vinasse and nitrogen fertilizers. *Frontiers in Microbiology*, *10*, 1680.

Deacon, G. B. (1978). Volatilization of methylmercuric chloride by hydrogen-sulfide. *Nature*, *275*, 344.

Dent, S. R., Beutel, M. W., Gantzer, P., & Moore, B. C. (2014). Response of methylmercury, total mercury, iron and manganese to oxygenation of an anoxic hypolimnion in north twin Lake, Washington. *Lake and Reservoir Management*, *30*, 119–130.

Driscoll, C. T., Blette, V., Yan, C., Schofield, C. L., Munson, R., & Holsapple, J. (1995). The role of dissolved organic-carbon in the chemistry and bioavailability of mercury in remote Adirondack Lakes. *Water, Air, and Soil Pollution*, *80*, 499–508.

Drott, A., Lambertsson, L., Bjorn, E., & Skyllberg, U. (2007). Importance of dissolved neutral mercury sulfides for methyl mercury production in contaminated sediments. *Environmental Science & Technology*, *41*, 2270–2276.

Du, H. X., Ma, M., Igarashi, Y., & Wang, D. Y. (2019). Biotic and abiotic degradation of methylmercury in aquatic ecosystems: A review. *Bulletin of Environmental Contamination and Toxicology*, *102*, 605–611.

Eagles-Smith, C. A., Silbergeld, E. K., Basu, N., Bustamante, P., Diaz-Barriga, F., Hopkins, W. A., et al. (2018). Modulators of mercury risk to wildlife and humans in the context of rapid global change. *Ambio*, *47*, 170–197.

Eckley, C. S., & Hintelmann, H. (2006). Determination of mercury methylation potentials in the water column of lakes across Canada. *Science of the Total Environment*, *368*, 111–125.

Ekstrom, E. B., & Morel, F. M. (2008). Cobalt limitation of growth and mercury methylation in sulfate-reducing bacteria. *Environmental Science & Technology*, *42*, 93–99.

Ekstrom, E. B., Morel, F. M., & Benoit, J. M. (2003). Mercury methylation independent of the acetyl-coenzyme a pathway in sulfate-reducing bacteria. *Applied and Environmental Microbiology*, *69*, 5414–5422.

Etique, M., Bouchet, S., Byrne, J. M., ThomasArrigo, L. K., Kaegi, R., & Kretzschmar, R. (2021). Mercury reduction by nanoparticulate vivianite. *Environmental Science & Technology*, *55*, 3399–3407.

Figueiredo, N., Serralheiro, M. L., Canario, J., Duarte, A., Hintelmann, H., & Carvalho, C. (2018). Evidence of mercury methylation and demethylation by the estuarine microbial communities obtained in stable Hg isotope studies. *International Journal of Environmental Research and Public Health*, *15*, 2141. https://doi.org/10.3390/ijerph15102141.

Fleming, E. J., Mack, E. E., Green, P. G., & Nelson, D. C. (2006). Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Applied and Environmental Microbiology*, *72*, 457–464.

Fossing, H., & Jørgensen, B. B. (1989). Measurement of bacterial sulfate reduction in sediments: Evaluation of a single-step chromium reduction method. *Biogeochemistry*, *8*, 205–222.

Fox, B., & Walsh, C. T. (1982). Mercuric reductase - purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulfide. *The Journal of Biological Chemistry*, *257*, 2498–2503.

Furukawa, K., Suzuki, T., & Tonomura, K. (1969). Decomposition of organic mercurial compounds by mercury-resistant bacteria. *Agricultural and Biological Chemistry Tokyo*, *33*, 128.

Gabaldon, T. (2005). Evolution of proteins and proteomes: A phylogenetics approach. *Evolutionary Bioinformatics*, *1*, 51–61.

Gadagkar, S. R., Rosenberg, M. S., & Kumar, S. (2005). Inferring species phylogenies from multiple genes: Concatenated sequence tree versus consensus gene tree. *The Journal of Experimental Zoology Part B*, *304b*, 64–74.

Gao, Y. S., Zhang, Z., Wu, J. W., Duan, L. H., Umar, A., Sun, L. Y., et al. (2013). A critical review on the heterogeneous catalytic oxidation of elemental mercury in flue gases. *Environmental Science & Technology*, *47*, 10813–10823.

Gilmour, C. C., Bullock, A. L., McBurney, A., Podar, M., & Elias, D. A. (2018). Robust mercury methylation across diverse methanogenic archaea. *MBio*, *9*, (e02403-02417).

Gilmour, C. C., Elias, D. A., Kucken, A. M., Brown, S. D., Palumbo, A. V., Schadt, C. W., et al. (2011). Sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 as a model for understanding bacterial mercury methylation. *Applied and Environmental Microbiology*, *77*, 3938–3951.

Gilmour, C. C., Henry, E. A., & Mithchell, R. (1992). Sulfate stimulation of mercury methylation in freshwater sediments. *Environmental Science & Technology*, *26*, 2281–2287.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., et al. (2013). Mercury methylation by novel microorganisms from new environments. *Environmental Science & Technology*, *47*, 11810–11820.

Gilmour, C. C., Riedel, G. S., Ederington, M. C., Bell, J. T., Benoit, J. M., Gill, G. A., et al. (1998). Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry*, *40*, 327–345.

Gilmour, C. C., Soren, A. B., Gionfriddo, C. M., Podar, M., Wall, J. D., Brown, S. D., et al. (2021). *Pseudodesulfovibrio mercurii* sp. nov., a mercury-methylating bacterium isolated from sediment. *International Journal of Systematic and Evolutionary Microbiology*, *71*(3). https://doi.org/10.1099/ijsem.0.004697.

Gionfriddo, C., Podar, M., Gilmour, C., Pierce, E., & Elias, D. (2019). *ORNL compiled mercury methylator database [online]. RNLCIFSFA (critical interfaces science focus area)*. Oak Ridge, Tennessee, USA: Oak Ridge National Lab. U.S. Department of Energy.

Gionfriddo, C. M., Stott, M. B., Power, J. F., Ogorek, J. M., Krabbenhoft, D. P., Wick, R., et al. (2020). Genome-resolved metagenomics and detailed geochemical speciation analyses yield new insights into microbial mercury cycling in geothermal springs. *Applied and Environmental Microbiology*, *86*, e00176–20. https://doi.org/10.1128/AEM.00176-20.

Gionfriddo, C. M., Wymore, A. M., Jones, D. S., Wilpiszeski, R. L., Lynes, M. M., Christensen, G. A., et al. (2020). An improved *hgcAB* primer set and direct high-throughput sequencing expand Hg-methylator diversity in nature. *Frontiers in Microbiology*, *11*, 541554.

Golding, G. R., Kelly, C. A., Sparling, R., Loewen, P. C., Rudd, J. W. M., & Barkay, T. (2002). Evidence for facilitated uptake of Hg(II) by *Vibrio anguillarum* and *Escherichia coli* under anaerobic and aerobic conditions. *Limnology and Oceanography*, *47*, 967–975.

Gordon, J., Quinton, W., Branfireun, B. A., & Olefeldt, D. (2016). Mercury and methylmercury biogeochemistry in a thawing permafrost wetland complex, Northwest Territories, Canada. *Hydrological Processes*, *30*, 3627–3638.

Graham, A. M., Aiken, G. R., & Gilmour, C. C. (2013). Effect of dissolved organic matter source and character on microbial Hg methylation in Hg-S-DOM solutions. *Environmental Science & Technology*, *47*, 5746–5754.

Graham, A. M., Bullock, A. L., Maizel, A. C., Elias, D. A., & Gilmour, C. C. (2012). Detailed assessment of the kinetics of Hg-cell association, Hg methylation, and methylmercury degradation in several *Desulfovibrio* species. *Applied and Environmental Microbiology*, *78*, 7337–7346.

Graham, A. M., Cameron-Burr, K. T., Hajic, H. A., Lee, C., Msekela, D., & Gilmour, C. C. (2017). Sulfurization of dissolved organic matter increases Hg-sulfide-dissolved organic matter bioavailability to a Hg-methylating bacterium. *Environmental Science & Technology*, *51*, 9080–9088.

Gray, J. E., & Hines, M. E. (2006). Mercury: Distribution, transport, and geochemical and microbial transformations from natural and anthropogenic sources - preface. *Applied Geochemistry*, *21*, 1819–1820.

Green, J., & Paget, M. S. (2004). Bacterial redox sensors. *Nature Reviews Microbiology*, *2*, 954–966.

Gregoire, D. S., Lavoie, N. C., & Poulain, A. J. (2018). Heliobacteria reveal fermentation as a key pathway for mercury reduction in anoxic environments. *Environmental Science & Technology*, *52*, 4145–4153.

Grégoire, D. S., & Poulain, A. J. (2016). A physiological role for Hg-II during phototrophic growth. *Nature Geoscience*, *9*, 121–125.

Grégoire, D. S., & Poulain, A. J. (2018). Shining light on recent advances in microbial mercury cycling. *FACETS*, *3*, 858–879.

Grossart, H. P., Massana, R., McMahon, K. D., & Walsh, D. A. (2020). Linking metagenomics to aquatic microbial ecology and biogeochemical cycles. *Limnology and Oceanography*, *65*, S2–S20.

Guzzi, G., Ronchi, A., & Pigatto, P. (2021). Toxic effects of mercury in humans and mammals. *Chemosphere*, *263*, 127990. https://doi.org/10.1016/j.chemosphere.2020.127990.

Ha, E., Basu, N., Bose-O'Reilly, S., Dorea, J. G., McSorley, E., Sakamoto, M., et al. (2017). Current progress on understanding the impact of mercury on human health. *Environmental Research*, *152*, 419–433.

Hamelin, S., Amyot, M., Barkay, T., Wang, Y., & Planas, D. (2011). Methanogens: Principal methylators of mercury in lake periphyton. *Environmental Science & Technology*, *45*, 7693–7700.

Harris-Hellal, J., Grimaldi, M., Garnier-Zarli, E., & Bousserrhine, N. (2011). Mercury mobilization by chemical and microbial iron oxide reduction in soils of French Guyana. *Biogeochemistry*, *103*, 223–234.

Hines, M. E., Faganeli, J., Adatto, I., & Horvat, M. (2006). Microbial mercury transformations in marine, estuarine and freshwater sediment downstream of the Idrija Mercury Mine, Slovenia. *Journal of Applied Geochemistry*, *21*, 1924–1939.

Hines, M. E., Horvat, M., Faganeli, J., Bonzongo, J. C. J., Barkay, T., Major, E. B., et al. (2000). Mercury biogeochemistry in the Idrija River, Slovenia, from above the mine into the Gulf of Trieste. *Environmental Research*, *83*, 129–139.

Hsu-Kim, H., Eckley, C. S., Acha, D., Feng, X., Gilmour, C. C., Jonsson, S., et al. (2018). Challenges and opportunities for managing aquatic mercury pollution in altered landscapes. *Ambio*, *47*, 141–169.

Hsu-Kim, H., Kucharzyk, K. H., Zhang, T., & Deshusses, M. A. (2013). Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: A critical review. *Environmental Science & Technology*, *47*, 2441–2456.

Hu, H. Y., Lin, H., Zheng, W., Rao, B., Feng, X. B., Liang, L. Y., et al. (2013). Mercury reduction and cell-surface adsorption by *Geobacter sulfurreducens* PCA. *Environmental Science & Technology*, *47*, 10922–10930.

Hu, H. Y., Lin, H., Zheng, W., Tomanicek, S. J., Johs, A., Feng, X. B., et al. (2013). Oxidation and methylation of dissolved elemental mercury by anaerobic bacteria. *Nature Geoscience*, *6*, 751–754.

Hu, H., Wang, B., Bravo, A. G., Bjorn, E., Skyllberg, U., Amouroux, D., et al. (2020). Shifts in mercury methylation across a peatland chronosequence: From sulfate reduction to methanogenesis and syntrophy. *Journal of Hazardous Materials*, *387*, 121967.

Huang, C. C., Narita, M., Yamagata, T., & Endo, G. (1999). Identification of three *merB* genes and characterization of a broad-spectrum mercury resistance module encoded by a class II transposon of *Bacillus megaterium* strain MB1. *Gene*, *239*, 361–366.

Huang, Z. S., Tan, X. Q., Wei, Z. S., Jiao, H. Y., Xiao, X. L., & Ming, S. (2020). Mercury oxidation coupled to autotrophic denitrifying branched sulfur oxidation and sulfur disproportionation for simultaneous removal of Hg(0) and NO. *Applied Microbiology and Biotechnology*, *104*, 8489–8504.

Imachi, H., Sekiguchi, Y., Kamagata, Y., Loy, A., Qiu, Y. L., Hugenholtz, P., et al. (2006). Non-sulfate-reducing, syntrophic bacteria affiliated with desulfotomaculum cluster I are widely distributed in methanogenic environments. *Applied and Environmental Microbiology*, *72*, 2080–2091.

Iwahori, K., Takeuchi, F., Kamimura, K., & Sugio, T. (2000). Ferrous iron-dependent volatilization of mercury by the plasma membrane of *Thiobacillus ferrooxidans*. *Applied and Environmental Microbiology*, *66*, 3823–3827.

Jensen, S., & Jernelov, A. (1969). Biological methylation of mercury in aquatic organisms. *Nature*, *223*, 753–754.

Jeremiason, J. D., Engstrom, D. R., Swain, E. B., Nater, E. A., Johnson, B. M., Almendinger, J. E., et al. (2006). Sulfate addition increases methylmercury production in an experimental wetland. *Environmental Science & Technology*, *40*, 3800–3806.

Jones, D. S., Walker, G. M., Johnson, N. W., Mitchell, C. P. J., Coleman Wasik, J. K., & Bailey, J. V. (2019). Molecular evidence for novel mercury methylating microorganisms in sulfate-impacted lakes. *The ISME Journal*, *13*(7), 1659–1675. https://doi.org/10.1038/s41396-019-0376-1.

Kang-Yun, C. S., Liang, X., Dershwitz, P., Gu, W., Schepers, A., Flatley, A., et al. (2022). Evidence for methanobactin "theft" and novel chalkophore production in methanotrophs: Impact on methanotrophic-mediated methylmercury degradation. *The ISME Journal*, *16*, 211–220.

Kanzler, C. R., Lian, P., Trainer, E. L., Yang, X. X., Govind, N., Parks, J. M., et al. (2018). Emerging investigator series: Methylmercury speciation and dimethylmercury production in sulfidic solutions. *Environmental Science: Processes & Impacts*, *20*, 584–594.

Kaschak, E., Knopf, B., Petersen, J. H., Bings, N. H., & Konig, H. (2014). Biotic methylation of mercury by intestinal and sulfate-reducing bacteria and their potential role in mercury accumulation in the tissue of the soil-living *Eisenia foetida*. *Soil Biology and Biochemistry*, *69*, 202–211.

Kelly, C. A., Rudd, J. W., & Holoka, M. H. (2003). Effect of pH on mercury uptake by an aquatic bacterium: Implications for Hg cycling. *Environmental Science & Technology*, *37*, 2941–2946.

Kerin, E. J., Gilmour, C. C., Roden, E., Suzuki, M. T., Coates, J. D., & Mason, R. P. (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Applied and Environmental Microbiology*, *72*, 7919–7921.

Khwaja, A. R., Bloom, P. R., & Brezonik, P. L. (2010). Binding strength of methylmercury to aquatic NOM. *Environmental Science & Technology*, *44*, 6151–6156.

Kielak, A. M., Barreto, C. C., Kowalchuk, G. A., van Veen, J. A., & Kuramae, E. E. (2016). The ecology of *Acidobacteria*: Moving beyond genes and genomes. *Frontiers in Microbiology*, *7*, 744. https://doi.org/10.3389/fmicb.2016.00744.

Kim, M., Han, S., Gieskes, J., & Deheyn, D. D. (2011). Importance of organic matter lability for monomethylmercury production in sulfate-rich marine sediments. *Science of the Total Environment*, *409*, 778–784.

King, J. K., Kostka, J. E., Frischer, M. E., & Saunders, F. M. (2000). Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. *Applied and Environmental Microbiology*, *66*, 2430–2437.

King, J. K., Saunders, F. M., Lee, R. F., & Jahnke, R. A. (1999). Coupling mercury methylation rates to sulfate reduction rates in marine sediments. *Environmental Toxicology and Chemistry*, *18*, 1362–1369.

Kip, N., Ouyang, W. J., van Winden, J., Raghoebarsing, A., van Niftrik, L., Pol, A., et al. (2011). Detection, isolation, and characterization of acidophilic methanotrophs from *Sphagnum* mosses. *Applied and Environmental Microbiology*, *77*, 5643–5654.

Kip, N., van Winden, J. F., Pan, Y., Bodrossy, L., Reichart, G. J., Smolders, A. J. P., et al. (2010). Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. *Nature Geoscience*, *3*, 617–621.

Klapstein, S. J., & O'Driscoll, N. J. (2018). Methylmercury biogeochemistry in freshwater ecosystems: A review focusing on DOM and photodemethylation. *Bulletin of Environmental Contamination and Toxicology*, *100*, 14–25.

Korthals, E. T., & Winfrey, M. R. (1987). Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. *Applied and Environmental Microbiology*, *53*, 2397–2404.

Kostal, J., Mulchandani, A., Gropp, K. E., & Chen, W. (2003). A temperature responsive biopolymer for mercury remediation. *Environmental Science & Technology*, *37*, 4457–4462.

Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019). RAxML-NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*, *35*, 4453–4455.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Kumari, S., Amit, Jamwal, R., Mishra, N., & Singh, D. K. (2020). Recent developments in environmental mercury bioremediation and its toxicity: A review. *Environmental Nanotechnology, Monitoring & Management*, *13*, 100283.

Kunapuli, U., Jahn, M. K., Lueders, T., Geyer, R., Heipieper, H. J., & Meckenstock, R. U. (2010). *Desulfitobacterium aromaticivorans* sp. nov. and *Geobacter toluenoxydans* sp. nov., iron-reducing bacteria capable of anaerobic degradation of monoaromatic hydrocarbons. *International Journal of Systematic and Evolutionary Microbiology*, *60*, 686–695.

Kwon, S. Y., Selin, N. E., Giang, A., Karplus, V. J., & Zhang, D. (2018). Present and future mercury concentrations in Chinese rice: Insights from modeling. *Global Biogeochemical Cycles*, *32*, 437–462.

Lafrance-Vanasse, J., Lefebvre, M., Di Lello, P., Sygusch, J., & Omichinski, J. G. (2009). Crystal structures of the organomercurial lyase MerB in its free and mercury-bound forms: Insights into the mechanisms of methylmercury degradation. *The Journal of Biological Chemistry*, *284*, 938–944.

Lamborg, C. H., Hansel, C. M., Bowman, K. L., Voelker, B. M., Marsico, R. M., Oldham, V. E., et al. (2021). Dark reduction drives evasion of mercury from the ocean. *Frontiers in Environmental Chemistry*, *2*, 659085. https://doi.org/10.3389/fenvc.2021.659085.

Larmola, T., Tuittila, E. S., Tiirola, M., Nykanen, H., Martikainen, P. J., Yrjala, K., et al. (2010). The role of *Sphagnum* mosses in the methane cycling of a boreal mire. *Ecology*, *91*, 2356–2365.

Latimer, W. M. (1952). *The oxidation states of the elements and their potentials in aqueous solutions* (2nd ed.). New York: Prentice-Hall.

Lei, P., Nunes, L. M., Liu, Y. R., Zhong, H., & Pan, K. (2019). Mechanisms of algal biomass input enhanced microbial Hg methylation in lake sediments. *Environment International*, *126*, 279–288.

Li, H. H., Mei, X. X., Liu, B. F., Xie, G. J., Ren, N. Q., & Xing, D. F. (2019). Quantitative proteomic analysis reveals the ethanologenic metabolism regulation of *Ethanoligenens harbinense* by exogenous ethanol addition. *Biotechnology for Biofuels*, *12*, 166. https://doi.org/10.1186/s13068-019-1511-y.

Lin, H. Y., Ascher, D. B., Myung, Y., Lamborg, C. H., Hallam, S. J., Gionfriddo, C. M., et al. (2021). Mercury methylation by metabolically versatile and cosmopolitan marine bacteria. *The ISME Journal*, *15*(6), 1810–1825. https://doi.org/10.1038/s41396-020-00889-4.

Lin, C.-C., Yee, N., & Barkay, T. (2012). Microbial transformations in the mercury cycle. In G. Liu, Y. Cai, & N. O'Driscoll (Eds.), *Environmental chemistry and toxicology of mercury* (pp. 155–191). John Wiley & Sons, Inc.

Liu, Z. C., Chen, B. N., Wang, L. A., Urbanovich, O., Nagorskaya, L., Li, X., et al. (2020). A review on phytoremediation of mercury contaminated soils. *Journal of Hazardous Materials*, *400*, 123138.

Liu, P., & Conrad, R. (2017). *Syntrophobacteraceae*-affiliated species are major propionate-degrading sulfate reducers in paddy soil. *Environmental Microbiology*, *19*, 1669–1686.

Liu, Y. R., Johs, A., Bi, L., Lu, X., Hu, H. W., Sun, D., et al. (2018). Unraveling microbial communities associated with methylmercury production in paddy soils. *Environmental Science & Technology*, *52*, 13110–13118.

Liu, Y. X., Liu, L., & Wang, Y. (2021). A critical review on removal of gaseous pollutants using sulfate radical-based advanced oxidation technologies. *Environmental Science & Technology*, *55*, 9691–9710.

Liu, Y. R., Yu, R. Q., Zheng, Y. M., & He, J. Z. (2014). Analysis of the microbial community structure by monitoring an Hg methylation gene (*hgcA*) in paddy soils along an Hg gradient. *Applied and Environmental Microbiology*, *80*, 2874–2879.

Lovley, D. R., & Phillips, E. J. P. (1987). Rapid assay for microbially reducible ferric iron in aquatic sediments. *Applied and Environmental Microbiology*, *53*, 1536–1540.

Lovley, D. R., & Walker, D. J. F. (2019). *Geobacter* protein nanowires. *Frontiers in Microbiology*, *10*, 2078. https://doi.org/10.3389/fmicb.2019.02078.

Lu, X., Gu, W., Zhao, L., Farhan Ul Haque, M., DiSpirito, A. A., Semrau, J. D., et al. (2017). Methylmercury uptake and degradation by methanotrophs. *Science Advances*, *3*, e1700041.

Lu, X., Liu, Y., Johs, A., Zhao, L., Wang, T., Yang, Z., et al. (2016). Anaerobic mercury methylation and demethylation by *Geobacter bemidjiensis* Bem. *Environmental Science & Technology*, *50*, 4366–4373.

Luo, H. W., Cheng, Q. Q., & Pan, X. L. (2020). Photochemical behaviors of mercury (Hg) species in aquatic systems: A systematic review on reaction process, mechanism, and influencing factor. *Science of the Total Environment*, *720*, 137540. https://doi.org/10.1016/j.scitotenv.2020.137540.

Ma, M., Du, H. X., & Wang, D. Y. (2019). Mercury methylation by anaerobic microorganisms: A review. *Critical Reviews in Environmental Science and Technology*, *49*, 1893–1936.

Madsen, E. L. (2011). Microorganisms and their roles in fundamental biogeochemical cycles. *Current Opinion in Biotechnology*, *22*, 456–464.

Marvin-DiPasquale, M. C., Agee, J. L., Bouse, R. M., & Jaffe, B. E. (2003). Microbial cycling of mercury in contaminated pelagic and wetland sediments of San Pablo Bay, California. *Environmental Geology*, *43*, 260–267.

Marvin-DiPasquale, M., Agee, J., McGowan, C., Oremland, R. S., Thomas, M., Krabbenhoft, D., et al. (2000). Methyl-mercury degradation pathways: A comparison among three mercury-impacted ecosystems. *Environmental Science & Technology*, *34*, 4908–4916.

Marvin-Dipasquale, M. C., & Oremland, R. S. (1998). Bacterial methylmercury degradation in Florida Everglades peat sediment. *Environmental Science & Technology*, *32*, 2556–2563.

Matsui, K., Yoshinami, S., Narita, M., Chien, M. F., Phung, L. T., Silver, S., et al. (2016). Mercury resistance transposons in *Bacilli* strains from different geographical regions. *FEMS Microbiology Letters*, *363*(5), fnw013. https://doi.org/10.1093/femsle/fnw013.

Matthews, D. A., Babcock, D. B., Nolan, J. G., Prestigiacomo, A. R., Effler, S. W., Driscoll, C. T., et al. (2013). Whole-lake nitrate addition for control of methylmercury in mercury-contaminated Onondaga lake, NY. *Environmental Research*, *125*, 52–60.

Mazrui, N. M., Jonsson, S., Thota, S., Zhao, J., & Mason, R. P. (2016). Enhanced availability of mercury bound to dissolved organic matter for methylation in marine sediments. *Geochimica et Cosmochimica Acta*, *194*, 153–162.

McCord, S. A., Beutel, M. W., Dent, S. R., & Schladow, S. G. (2016). Evaluation of mercury cycling and hypolimnetic oxygenation in mercury-impacted seasonally stratified reservoirs in the Guadalupe River watershed, California. *Water Resources Research*, *52*, 7726–7743.

McDaniel, E. A., Peterson, B. D., Stevens, S. L. R., Tran, P. Q., Anantharaman, K., & McMahon, K. D. (2020). Expanded phylogenetic diversity and metabolic flexibility of mercury-methylating microorganisms. *mSystems*, *5*, (e00299-00220).

McInerney, M. J., Sieber, J. R., & Gunsalus, R. P. (2011). Microbial syntrophy: Ecosystem-level biochemical cooperation. *Microbe*, *6*, 479–485.

McInerney, M. J., Struchtemeyer, C. G., Sieber, J., Mouttaki, H., Stams, A. J., Schink, B., et al. (2008). Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Annals of the New York Academy of Sciences*, *1125*, 58–72.

Mehrotra, A. S., Horne, A. J., & Sedlak, D. L. (2003). Reduction of net mercury methylation by iron in *Desulfobulbus propionicus* (1pr3) cultures: Implications for engineered wetlands. *Environmental Science & Technology*, *37*, 3018–3023.

Mehrotra, A. S., & Sedlak, D. L. (2005). Decrease in net mercury methylation rates following iron amendment to anoxic wetland sediment slurries. *Environmental Science & Technology*, *39*, 2564–2570.

Melnick, J. G., & Parkin, G. (2007). Cleaving mercury-alkyl bonds: A functional model for mercury detoxification by MerB. *Science*, *317*, 225–227.

Merritt, K. A., & Amirbahman, A. (2008). Methylmercury cycling in estuarine sediment pore waters (Penobscot River estuary, Maine, USA). *Limnology and Oceanography*, *53*, 1064–1075.

Miller, S. M. (2007). Cleaving C-Hg bonds: Two thiolates are better than one. *Nature Chemical Biology*, *3*, 537–538.

Morel, F. M. M., Kraepiel, A. M. L., & Amyot, M. (1998). The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics*, *29*, 543–566.

Morris, B. E., Henneberger, R., Huber, H., & Moissl-Eichinger, C. (2013). Microbial syntrophy: Interaction for the common good. *FEMS Microbiology Reviews*, *37*, 384–406.

Munch, J. C., & Ottow, J. C. G. (1983). Reductive transformation mechanism of ferric oxides in hydromorphic soils. *Ecological Bulletins*, *35*, 383–394.

Muyzer, G., & Stams, A. J. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews. Microbiology*, *6*, 441–454.

Naguib, M. M., El-Gendy, A. O., & Khairalla, A. S. (2018). Microbial diversity of *mer* operon genes and their potential rules in mercury bioremediation and resistance the open. *Biotechnology Journal*, *12*, 56–77.

Nazaret, S., Jeffrey, W. H., Saouter, E., Von Haven, R., & Barkay, T. (1994). *merA* gene expression in aquatic environments measured by mRNA production and Hg(II) volatilization. *Applied and Environmental Microbiology*, *60*, 4059–4065.

Nriagu, J. O. (1994). Mechanistic steps in the photoreduction of mercury in natural-waters. *Science of the Total Environment*, *154*, 1–8.

Obrist, D., Agnan, Y., Jiskra, M., Olson, C. L., Colegrove, D. P., Hueber, J., et al. (2017). Tundra uptake of atmospheric elemental mercury drives arctic mercury pollution. *Nature*, *547*, 201.

Obrist, D., Kirk, J. L., Zhang, L., Sunderland, E. M., Jiskra, M., & Selin, N. E. (2018). A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. *Ambio*, *47*, 116–140.

Orem, W. H., Fitz, C., Krabbenhoft, D. P., Poulin, B. A., Varonkal, M. S., & Aiken, G. R. (2020). Ecosystem-scale modeling and field observations of sulfate and methylmercury distributions in the Florida Everglades: Responses to reductions in sulfate loading. *Aquatic Geochemistry*, *26*, 191–220.

Oremland, R. S., & Capone, D. G. (1988). Use of specific inhibitors in biogeochemistry and microbial ecology. *Advances in Microbial Ecology*, *10*, 285–383.

Oremland, R. S., Culbertson, C. W., & Winfrey, M. R. (1991). Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation. *Applied and Environmental Microbiology*, *57*, 130–137.

Oremland, R. S., Miller, L. G., Dowdle, P., Connell, T., & Barkay, T. (1995). Methylmercury oxidative-degradation potentials in contaminated and pristine sediments of the Carson River, Nevada. *Applied and Environmental Microbiology*, *61*, 2745–2753.

Pacyna, J. M. (2020). Recent advances in mercury research. *Science of the Total Environment*, *738*, 139955. https://doi.org/10.1016/j.scitotenv.2020.139955.

Pak, K., & Bartha, R. (1998a). Mercury methylation by interspecies hydrogen and acetate transfer between sulfidogens and methanogens. *Applied and Environmental Microbiology*, *64*, 1987–1990.

Pak, K., & Bartha, R. (1998b). Products of mercury demethylation by sulfidogens and methanogens. *Bulletin of Environmental Contamination and Toxicology*, *61*, 690–694.

Pak, K. R., & Bartha, R. (1998c). Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. *Applied and Environmental Microbiology*, *64*, 1013–1017.

Paranjape, A. R., & Hall, B. D. (2017). Recent advances in the study of mercury methylation in aquatic systems. *FACETS*, *2*, 85–119. https://doi.org/10.1139/facets-2016-0027.

Parks, J. M., Guo, H., Momany, C., Liang, L., Miller, S. M., Summers, A. O., et al. (2009). Mechanism of Hg-C protonolysis in the organomercurial lyase MerB. *Journal of the American Chemical Society*, *131*, 13278–13285.

Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Jr., Smith, S. D., et al. (2013). The genetic basis for bacterial mercury methylation. *Science*, *339*, 1332–1335.

Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., et al. (2020). Mercury methylation genes identified across diverse anaerobic microbial guilds in a eutrophic sulfate-enriched lake. *Environmental Science & Technology*, *54*, 15840–15851.

Pickhardt, P. C., Folt, C. L., Chen, C. Y., Klaue, B., & Blum, J. D. (2002). Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 4419–4423.

Pitts, K. E., & Summers, A. O. (2002). The roles of thiols in the bacterial organomercurial lyase (MerB). *Biochemistry*, *41*, 10287–10296. https://doi.org/10.1021/bi0259148.

Plugge, C. M., Zhang, W., Scholten, J. C., & Stams, A. J. (2011). Metabolic flexibility of sulfate-reducing bacteria. *Frontiers in Microbiology*, *2*, 81. https://doi.org/10.3389/fmicb.2011.00081.

Podar, M., Gilmour, C. C., Brandt, C. C., Soren, A., Brown, S. D., Crable, B. R., et al. (2015). Global prevalence and distribution of genes and microorganisms involved in mercury methylation. *Science Advances*, *1*, e1500675.

Poulain, A. J., Amyot, M., Findlay, D., Telor, S., Barkay, T., & Hintelmann, H. (2004). Biological and photochemical production of dissolved gaseous mercury in a boreal lake. *Limnology and Oceanography*, *49*, 2265–2275.

Poulain, A. J., & Barkay, T. (2013). Cracking the mercury methylation code. *Science*, *339*, 1280–1281.

Priyadarshanee, M., Chatterjee, S., Rath, S., Dash, H. R., & Das, S. (2022). Cellular and genetic mechanism of bacterial mercury resistance and their role in biogeochemistry and bioremediation. *Journal of Hazardous Materials*, *423*(Pt A), 126985. https://doi.org/10.1016/j.jhazmat.2021.126985.

Probst, A. J., Ladd, B., Jarett, J. K., Geller-McGrath, D. E., Sieber, C. M. K., Emerson, J. B., et al. (2018). Differential depth distribution of microbial function and putative symbionts through sediment- hosted aquifers in the deep terrestrial subsurface. *Nature Microbiology*, *3*, 328–336.

Qian, C., Chen, H. M., Johs, A., Lu, X., An, J., Pierce, E. M., et al. (2018). Quantitative proteomic analysis of biological processes and responses of the bacterium *Desulfovibrio desulfuricans* ND132 upon deletion of its mercury methylation genes. *Proteomics*, *18*, 1700479. https://doi.org/10.1002/pmic.201700479.

Raj, D., & Maiti, S. K. (2019). Sources, toxicity, and remediation of mercury: An essence review. *Environmental Monitoring and Assessment*, *191*(9), 566. https://doi.org/10.1007/s10661-019-7743-2.

Ralston, D. M., & O'Halloran, T. V. (1990). Ultrasensitivity and heavy-metal selectivity of the allosterically modulated MerR transcription complex. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 3846–3850.

Ramlal, P. S., Rudd, J. W. M., & Hecky, R. E. (1986). Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. *Applied and Environmental Microbiology*, *51*, 110–114.

Ranchou-Peyruse, M., Monperrus, M., Bridou, R., Duran, R., Amouroux, D., Salvado, J. C., et al. (2009). Overview of mercury methylation capacities among anaerobic bacteria including representatives of the sulphate-reducers: Implications for environmental studies. *Geomicrobiology Journal*, *26*, 1–8.

Ravel, J., DiRuggiero, J., Robb, F. T., & Hill, R. T. (2000). Cloning and sequence analysis of the mercury resistance operon of *Streptomyces* sp. strain CHR28 reveals a novel putative second regulatory gene. *Journal of Bacteriology*, *182*, 2345–2349.

Regnell, O., & Watras, C. J. (2019). Microbial mercury methylation in aquatic environments: A critical review of published field and laboratory studies. *Environmental Science & Technology*, *53*, 4–19.

Roden, E. E., & Lovley, D. R. (1993). Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Applied and Environmental Microbiology*, *59*, 734–742.

Rolfhus, K. R., & Fitzgerald, W. F. (2004). Mechanisms and temporal variability of dissolved gaseous mercury production in coastal seawater. *Marine Chemistry*, *90*, 125–136.

Roth, S., Poulin, B. A., Baumann, Z., Liu, X., Zhang, L., Krabbenhoft, D. P., et al. (2021). Nutrient inputs stimulate mercury methylation by syntrophs in a subarctic peatland. *Frontiers in Microbiology*, *12*, 741523. https://doi.org/10.3389/fmicb.2021.741523.

Rothenberg, S. E., Windham-Myers, L., & Creswell, J. E. (2014). Rice methylmercury exposure and mitigation: A comprehensive review. *Environmental Research*, *133*, 407–423.

Rowland, I. R., Davies, M. J., & Grasso, P. (1977). Volatilisation of methylmercuric chloride by hydrogen sulphide. *Nature*, *265*, 718–719.

Sall, M. L., Diaw, A. K. D., Gningue-Sall, D., Aaron, S. E., & Aaron, J. J. (2020). Toxic heavy metals: Impact on the environment and human health, and treatment with conducting organic polymers, a review. *Environemental Science and Pollution Research*, *27*, 29927–29942.

Saouter, E., Gillman, M., & Barkay, T. (1995). An evaluation of mer-specified reduction of ionic mercury as a remedial tool of a mercury-contaminated fresh-water pond. *Journal of Industrial Microbiology*, *14*, 343–348.

Schaefer, J. K., Kronberg, R. M., Bjorn, E., & Skyllberg, U. (2020). Anaerobic guilds responsible for mercury methylation in boreal wetlands of varied trophic status serving as either a methylmercury source or sink. *Environmental Microbiology*, *22*, 3685–3699.

Schaefer, J. K., Kronberg, R. M., Morel, F. M., & Skyllberg, U. (2014). Detection of a key Hg methylation gene, *hgcA*, in wetland soils. *Environmental Microbiology Reports*, *6*, 441–447.

Schaefer, J. K., Rocks, S. S., Zheng, W., Liang, L. Y., Gu, B. H., & Morel, F. M. M. (2011). Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 8714–8719.

Schaefer, J. K., Yagi, J., Reinfelder, J. R., Cardona, T., Ellickson, K. M., Tel-Or, S., et al. (2004). Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury-contaminated natural waters. *Environmental Science & Technology*, *38*, 4304–4311.

Schottel, J., Mandal, A., Clark, D., Silver, S., & Hedges, R. W. (1974). Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. *Nature*, *251*, 335–337.

Schuur, E. A. G., & Mack, M. C. (2018). Ecological response to permafrost thaw and consequences for local and global ecosystem services. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 279–301.

Sellers, P., Kelly, C. A., Rudd, J. W. M., & MacHutchon, A. R. (1996). Photodegradation of methylmercury in lakes. *Nature*, *380*, 694–697.

Shirokova, L. S., Chupakov, A. V., Ivanova, I. S., Moreva, O. Y., Zabelina, S. A., Shutskiy, N. A., et al. (2021). Lichen, moss and peat control of C, nutrient and trace metal regime in lakes of permafrost peatlands. *Science of the Total Environment*, *782*, 146737. https://doi.org/10.1016/j.scitotenv.2021.146737.

Skyllberg, U. (2008). Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: Illumination of controversies and implications for MeHg net production. *Journal of Geophysical Research – Biogeosciences*, 113. G2. https://doi.org/10.1029/2008JG000745.

Smith, S. D., Bridou, R., Johs, A., Parks, J. M., Elias, D. A., Hurt, R. A., Jr., et al. (2015). Site-directed mutagenesis of HgcA and HgcB reveals amino acid residues important for mercury methylation. *Applied and Environmental Microbiology*, *81*, 3205–3217.

Smith, T., Pitts, K., McGarvey, J. A., & Summers, A. O. (1998). Bacterial oxidation of mercury metal vapor, Hg(0). *Applied and Environmental Microbiology*, *64*, 1328–1332.

Sorokin, D. Y., Tourova, T. P., Mussmann, M., & Muyzer, G. (2008). *Dethiobacter alkaliphilus* gen. nov. sp. nov., and *Desulfurivibrio alkaliphilus* gen. nov. sp. nov.: Two novel representatives of reductive sulfur cycle from soda lakes. *Extremophiles*, *12*, 431–439.

Spangler, W. J., Spigarelli, J. L., Rose, J. M., Flippin, R. S., & Miller, H. H. (1973). Degradation of methylmercury by bacteria isolated from environmental samples. *Applied Microbiology*, *25*, 488–493.

Spangler, W. J., Spigarelli, J. L., Rose, J. M., & Miller, H. M. (1973). Methylmercury - bacterial degradation in lake sediments. *Science*, *180*, 192–193.

Spring, S., Sorokin, D. Y., Verbarg, S., Rohde, M., Woyke, T., & Kyrpides, N. C. (2019). Sulfate-reducing bacteria that produce exopolymers thrive in the calcifying zone of a hypersaline cyanobacterial mat. *Frontiers in Microbiology*, *10*, 862. https://doi.org/10.3389/fmicb.2019.00862.

Stackebrandt, E. (2014). The emended family *Peptococcaceae* and description of the families *Desulfitobacteriaceae*, *Desulfotomaculaceae*, and *Thermincolaceae*. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The prokaryotes* (pp. 285–290). Berlin, Heidelberg: Springer.

Stackebrandt, E., Sproer, C., Rainey, F. A., Burghardt, J., Pauker, O., & Hippe, H. (1997). Phylogenetic analysis of the genus *Desulfotomaculum*: Evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. *International Journal of Systematic Bacteriology*, *47*, 1134–1139.

Strong, P. J., Xie, S., & Clarke, W. P. (2015). Methane as a resource: Can the methanotrophs add value? *Environmental Science & Technology*, *49*, 4001–4018.

Sugio, T., Iwahori, K., Takeuchi, F., Negishi, A., Maeda, T., & Kamimura, K. (2001). Cytochrome c oxidase purified from a mercury-resistant strain of *Acidithiobacillus ferrooxidans* volatilizes mercury. *Journal of Bioscience and Bioengineering*, *92*, 44–49.

Summers, A. O. (1986). Organization, expression, and evolution of genes for mercury resistance. *Annual Review of Microbiology*, *40*, 607–634.

Summers, A. O. (1992). Untwist and shout - a heavy metal-responsive transcriptional regulator. *Journal of Bacteriology*, *174*, 3097–3101.

Takeuchi, F., Iwahori, K., Kamimura, K., Negishi, A., Maeda, T., & Sugio, T. (2001). Volatilization of mercury under acidic conditions from mercury-polluted soil by a mercury-resistant *Acidithiobacillus ferrooxidans* SUG 2-2. *Bioscience Biotechnology and Biochemistry*, *65*, 1981–1986.

Takeuchi, F., Iwahori, K., Kamimura, K., & Sugio, T. (1999). Isolation and some properties of *Thiobacillus ferrooxidans* strains with differing levels of mercury resistance from natural environments. *Journal of Bioscience and Bioengineering*, *88*, 387–392.

Tang, W. L., Liu, Y. R., Guan, W. Y., Zhong, H., Qu, X. M., & Zhang, T. (2020). Understanding mercury methylation in the changing environment: Recent advances in assessing microbial methylators and mercury bioavailability. *Science of the Total Environment*, *714*, 136827.

Tarbier, B., Hugelius, G., Sannel, A. B. K., Baptista-Salazar, C., & Jonsson, S. (2021). Permafrost thaw increases methylmercury formation in subarctic Fennoscandia. *Environmental Science & Technology*, *55*, 6710–6717.

Taubner, L. M., McGuirl, M. A., Dooley, D. M., & Copie, V. (2006). Structural studies of apo NosL, an accessory protein of the nitrous oxide reductase system: Insights from structural homology with MerB, a mercury resistance protein. *Biochemistry*, *45*, 12240–12252.

Todorova, S. G., Driscoll, C. T., Matthews, D. A., Effler, S. W., Hines, M. E., & Henry, E. A. (2009). Evidence for regulation of monomethyl mercury by nitrate in a seasonally stratified, eutrophic lake. *Environmental Science & Technology*, *43*, 6572–6578.

Tomiyasu, T., Kodamatani, H., Imura, R., Matsuyama, A., Miyamoto, J., Akagi, H., et al. (2017). The dynamics of mercury near Idrija mercury mine, Slovenia: Horizontal and vertical distributions of total, methyl, and ethyl mercury concentrations in soils. *Chemosphere*, *184*, 244–252.

Toribara, T. Y., Shields, C. P., & Koval, L. (1970). Behaviour of dilute solutions of mercury. *Talanta*, *17*, 1025–1028.

Tsui, M. T. K., Blum, J. D., & Kwon, S. Y. (2020). Review of stable mercury isotopes in ecology and biogeochemistry. *Science of the Total Environment*, *716*, 135386. https://doi.org/10.1016/j.scitotenv.2019.135386.

Velasquez-Riano, M., & Benavides-Otaya, H. D. (2016). Bioremediation techniques applied to aqueous media contaminated with mercury. *Critical Reviews in Biotechnology*, *36*, 1124–1130.

Villar, E., Cabrol, L., & Heimburger-Boavida, L. E. (2020). Widespread microbial mercury methylation genes in the global ocean. *Environmental Microbiology Reports*, *12*, 277–287.

Vitt, D. H., Halsey, L. A., & Zoltai, S. C. (1994). The bog landforms of continental western Canada in relation to climate and permafrost patterns. *Arctic and Alpine Research*, *26*, 1–13.

Vorobev, A., Jagadevan, S., Baral, B. S., DiSpirito, A. A., Freemeier, B. C., Bergman, B. H., et al. (2013). Detoxification of mercury by methanobactin from *Methylosinus trichosporium* OB3b. *Applied and Environmental Microbiology*, *79*, 5918–5926.

Wagner-Dobler, I. (2003). Pilot plant for bioremediation of mercury-containing industrial wastewater. *Applied Microbiology and Biotechnology*, *62*, 124–133.

Waite, D. W., Chuvochina, M., Pelikan, C., Parks, D. H., Yilmaz, P., Wagner, M., et al. (2020). Proposal to reclassify the proteobacterial classes *Deltaproteobacteria* and *Oligoflexia*, and the phylum *Thermodesulfobacteria* into four phyla reflecting major functional capabilities. *International Journal of Systematic and Evolutionary Microbiology*, *70*, 5972–6016.

Wallrabenstein, C., Hauschild, E., & Schink, B. (1994). Pure culture and cytological properties of *Syntrophobacter wolinii*. *FEMS Microbiology Letters*, *123*, 249–254.

Wang, Y. W., Bartov, G., Wang, T., Reinfelder, J. R., Johnson, T. M., & Yee, N. (2021). Rapid attainment of isotopic equilibrium after mercury reduction by ferrous iron minerals and isotopic exchange between Hg(II) and Hg(0). *ACS Earth and Space Chemistry*, *5*, 1384–1394.

Wang, Y., Schaefer, J. K., Mishra, B., & Yee, N. (2016). Intracellular Hg(0) oxidation in *Desulfovibrio desulfuricans* ND132. *Environmental Science & Technology*, *50*, 11049–11056.

Warner, K. A., Roden, E. E., & Bonzongo, J. C. (2003). Microbial mercury transformation in anoxic freshwater sediments under iron-reducing and other electron-accepting conditions. *Environmental Science & Technology*, *37*, 2159–2165.

Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M., & Barton, G. J. (2009). Jalview version 2-a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, *25*, 1189–1191.

Watras, C. J., Morrison, K. A., Host, J. S., & Bloom, N. S. (1995). Concentration of mercury species in relationship to other site-specific factors in the surface saters of northern Wisconsin Lakes. *Limnology and Oceanography*, *40*, 556–565.

Weber, J. H. (1993). Review of possible paths for abiotic methylation of mercury(ii) in the aquatic environment. *Chemosphere*, *26*, 2063–2077.

Weiss, A. A., Murphy, S. D., & Silver, S. (1977). Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. *Journal of Bacteriology*, *132*, 197–208.

West, J., Graham, A. M., Van, L. N., & Jonsson, S. (2020). Dimethylmercury degradation by dissolved sulfide and mackinawite. *Environmental Science & Technology*, *54*, 13731–13738.

Wiatrowski, H. A., Das, S., Kukkadapu, R., Ilton, E. S., Barkay, T., & Yee, N. (2009). Reduction of Hg(II) to Hg(0) by magnetite. *Environmental Science & Technology*, *43*, 5307–5313.

Wiatrowski, H. A., Ward, P. M., & Barkay, T. (2006). Novel reduction of mercury(II) by mercury-sensitive dissimilatory metal reducing bacteria. *Environmental Science & Technology*, *40*, 6690–6696.

Wilkins, M. J., Livens, F. R., Vaughan, D. J., & Lloyd, J. R. (2006). The impact of Fe(III)-reducing bacteria on uranium mobility. *Biogeochemistry*, *78*, 125–150.

Winderl, C., Penning, H., Netzer, F., Meckenstock, R. U., & Lueders, T. (2010). DNA-SIP identifies sulfate-reducing *Clostridia* as important toluene degraders in tar-oil-contaminated aquifer sediment. *The ISME Journal*, *4*, 1314–1325.

Wood, J. M., Kennedy, F. S., & Rosen, C. G. (1968). Synthesis of methyl-mercury compounds by extracts of a methanogenic bacterium. *Nature*, *220*, 173–174.

Wu, Q., Hu, H., Meng, B., Wang, B., Poulain, A. J., Zhang, H., et al. (2020). Methanogenesis is an important process in controlling MeHg concentration in rice paddy soils affected by mining activities. *Environmental Science & Technology*, *54*, 13517–13526.

Wu, G., Zhao, N., Zhang, C., Lam, Y. Y., & Zhao, L. (2021). Guild-based analysis for understanding gut microbiome in human health and diseases. *Genome Medicine*, *13*, 22.

Xing, Y., Yan, B., Lu, P., Cui, X., Li, L., & Wang, M. (2017). Purification of Hg(0) from flue gas by wet oxidation method and its mechanism: A review. *Environmental Science and Pollution Research International*, *24*, 26310–26323.

Xu, J., Buck, M., Eklof, K., Ahmed, O. O., Schaefer, J. K., Bishop, K., et al. (2019). Mercury methylating microbial communities of boreal forest soils. *Scientific Reports*, *9*, 518.

Yang, L. X., Zhang, Y. Y., Wang, F. F., Luo, Z. D. E., Guo, S. J., & Strahle, U. (2020). Toxicity of mercury: Molecular evidence. *Chemosphere*, 245, 125586. https://doi.org/10.1016/j.chemosphere.2019.125586.

Yu, R. Q. (2011). *Microbial mercury methylation and demethylation: Biogeochemical mechanisms and metagenomic perspectives in freshwater ecosystems*. *PhD Thesis* (pp. 1–206). New Brunswick: Department of Biochemistry and Microbiology, Rutgers University.

Yu, R. Q., Adatto, I., Montesdeoca, M. R., Driscoll, C. T., Hines, M. E., & Barkay, T. (2010). Mercury methylation in *Sphagnum* moss mats and its association with sulfate-reducing bacteria in an acidic Adirondack forest lake wetland. *FEMS Microbiology Ecology*, *74*, 655–668.

Yu, R. Q., Flanders, J. R., Mack, E. E., Turner, R., Mirza, M. B., & Barkay, T. (2012). Contribution of coexisting sulfate and iron reducing bacteria to methylmercury production in freshwater river sediments. *Environmental Science & Technology*, *46*, 2684–2691.

Yu, R. Q., Reinfelder, J. R., Hines, M. E., & Barkay, T. (2013). Mercury methylation by the methanogen *Methanospirillum hungatei*. *Applied and Environmental Microbiology*, *79*, 6325–6330.

Yu, R. Q., Reinfelder, J. R., Hines, M. E., & Barkay, T. (2018). Syntrophic pathways for microbial mercury methylation. *The ISME Journal*, *12*, 1826–1835.

Yu, H., Susanti, D., McGlynn, S. E., Skennerton, C. T., Chourey, K., Iyer, R., et al. (2018). Comparative genomics and proteomic analysis of assimilatory sulfate reduction pathways in anaerobic methanotrophic archaea. *Frontiers in Microbiology*, *9*, 2917.

Zhao, L., Chen, H., Lu, X., Lin, H., Christensen, G. A., Pierce, E. M., et al. (2017). Contrasting effects of dissolved organic matter on mercury methylation by *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132. *Environmental Science & Technology*, *51*, 10468–10475.

Zhou, Z., Liu, Y., Xu, W., Pan, J., Luo, Z. H., & Li, M. (2020). Genome- and community-level interaction insights into carbon utilization and element cycling functions of hydrothermarchaeota in hydrothermal sediment. *mSystems*, *5*(1), e00795–19. https://doi.org/10.1128/mSystems.00795-19.