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Research Article

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Abstract

Mercury (Hg) is a global pollutant of environmental and health concern; its methylated form, methylmercury (MeHg) is a potent neurotoxin. Sulfur-containing molecules play a role in MeHg production by microorganisms. While sulfides are considered to limit Hg methylation, sulfate and cysteine were shown to favor this process. However, these two forms can be endogenously converted by microorganisms into sulfide. Here, we explore the effect of sulfide (produced by the cell or supplied exogenously) on Hg methylation. For this purpose, *Pseudodesulfovibrio hydrargyri* BerOc1 was cultivated in non-sulfidogenic conditions with addition of cysteine and sulfide as well as in sulfidogenic conditions. We report that Hg methylation depends on sulfide concentration in the culture rather than on the initial form of sulfur (cysteine, sulfide or sulfate) added, and was independent of hgcA expression. Interestingly, MeHg production was maximal at 0.1-0.5 mM of sulfides. Besides, a strong positive correlation between MeHg in the extracellular medium and the increase of sulfide concentrations was observed, suggesting a facilitated MeHg export with sulfide and/or higher desorption from the cell. We demonstrate that sulfides (exogenous or endogenous) play a key role in controlling mercury methylation, and should be considered when investigating the impact of Hg on natural environments.

Introduction

Mercury (Hg) is of high concern due to its toxicity even at low concentration. It is persistent, highly volatile and is able to convert into highly toxic methylmercury (CH_3Hg or MeHg), a strong neurotoxin, that is bioaccumulated and biomagnified across the aquatic food web (Atchison and Hare 1994; Boudou and Ribeyre 1997; Farina et al. 2011; Rice et al. 2014). Biotic methylation of inorganic mercury (Hg(II)) by microorganisms is the primary source of MeHg (Jensen and Jernelöv 1969). The environmental and health risk associated to Hg contamination is highly dependent on environmental parameters that can either promote or limit the production of MeHg by microorganisms at the bottom of the food web. Current knowledge on Hg methylation is based on anaerobic microorganisms such as sulfate-reducing (SRB) (Compeau and Bartha) and iron-reducing bacteria (Bravo et al. 2018; Kerin et al. 2006) although other microbial groups (Gilmour et al. 2013), like methanogens, are also known to methylate Hg. Two genes encoding HgcA (a putative methyl transferase involved in methyl transfer to Hg(II)) and HgcB (a Fe-S cluster protein involved in HgcA electron recycling) are required for Hg methylation in these microorganisms (Parks et al. 2013). However, no link between the expression of these genes and the presence of Hg(II) has been so far observed (Goñi-Urriza et al. 2015; Qian et al. 2018).

In SRB, Hg methylation was shown to be higher under non-sulfide producing growth metabolism (non-sulfidogenic growth: fumarate respiration or fermentation) compared to sulfide producing condition (sulfidogenic growth: sulfate respiration) (Bridou et al. 2011; Gilmour et al. 2011; Goñi-Urriza et al. 2015). A negative linear correlation between sulfide and Hg methylation was also observed in a millimolar range of sulfide (Benoit et al. 2001a). The decrease of Hg methylation was attributed to a variation in predicted Hg-S complexes, specifically to a decrease of the dissolved neutral Hg-S species (HgS^0 and $Hg(SH)_2^0$) (Benoit et al. 1999a, b, 2001b; Drott et al. 2007). These neutral forms are suspected to be more available

for the cell than the charged species and their decrease negatively affect the Hg methylation. While, it is accepted that in SRB sulfides inhibit Hg methylation, it was observed recently in methanogens that sulfides at low concentration may promote mercury methylation (Gilmour et al. 2018), questioning the effect of sulfides concentration as variable on Hg methylation.

The effect of thiols on Hg methylation has been also investigated (Adediran et al. 2019; Liu et al. 2016; Schaefer and Morel 2009; Thomas et al. 2020), although they are present at much lower concentrations in aquatic environments compared to sulfate/sulfide (Bouchet et al. 2018; Liem-Nguyen et al. 2017). Among these thiols, cysteine (at concentrations between 1 and 100 µM) increased the production of MeHg (Schaefer and Morel 2009). However, cysteine degradation by microbes produces sulfides (Graham et al. 2012a, b; Thomas and Gaillard 2017; Thomas et al. 2018). These produced sulfides are expected to limit Hg methylation, although they can form Hg-S complexes that favor Hg bio-uptake (Thomas and Gaillard 2017; Thomas et al. 2018). The effect of cysteine on Hg methylation is not yet fully understood. The sulfides produced by SRB from cysteine degradation (and not cysteine *per se*) could be responsible for the observed increase in Hg methylation.

Our hypothesis is that Hg(II) methylation is modulated by sulfide regardless of its origin; environmental or produced by the bacteria from cysteine or sulfate. However, whether sulfides enhance or inhibit Hg methylation may depends on the concentration of these molecules present in the cell environment. To support this hypothesis, in this work, (i) the potentials and rates of Hg methylation (ii) the partitioning between extracellular and cell-associated fractions of Hg species (to distinguish exported from the adsorbed/intracellular MeHg and Hg(II)) and, (iii) the *hgcA* expression were measured in the SRB *Pseudodesulfovibrio hydrargyri* BerOc1 grown with different concentrations of added (exogenous) or produced (endogenous) sulfides along with various Hg concentrations. For this purpose, we used *P. hydrargyri* BerOc1 under three different conditions regarding sulfide contents: grown i) under fumarate respiration (non sulfidogenic metabolism) in the presence of a wide range of exogenous sulfide concentrations, ii) under fumarate respiration in the presence of cysteine and, iii) under sulfate reduction (sulfidogenic metabolism). Because Hg methylation depends on Hg(II) concentration (Gilmour et al. 2011; Isaure et al. 2020), the effect of sulfides (added or produced) was also examined at three different Hg(II) concentrations (from nM to µM). Our study provides new insights into the major role of sulfides on Hg methylation process at sulfide concentrations of environmental relevance, which is important to address the risks of Hg regarding the environmental and the health standpoints on this issue.

Materials And Methods

Culture medium composition and growth conditions

The SRB *Pseudodesulfovibrio hydrargyri* strain BerOc1 was isolated from the Berre Lagoon sediments and it has been investigated for more than one decade for its capacity to methylate Hg (Ranchou-Peyruse et al. 2004, 2009, 2018). To perform all the experiments, the strain was grown anaerobically in the dark, at 37°C and pH 7.0 in the Brackish Multipurpose Medium (Widdel and Bak 1992) containing (per liter): 10 g

NaCl, 1.2 g $MgCl_2$•$6H_2O$, 0.1 g $CaCl_2$•$2H_2O$, 0.25 g NH_4Cl, 0.5 g KCl, 1 mL trace metal elements SL12B (Overmann et al.), 1 mL Selenite-Tungstate, 2.38 g HEPES, 1 mL V7 vitamins solution (Trüper and Pfennig 1992) and 0.2 g KH_2PO_4. Selenite-Tungstate solution is composed of 0.5 g NaOH, 2 mg Na_2SeO_3 and 4 mg $Na_2WO_4.2H_2O$ per liter and SL12B solution is composed of 300 mg H_3BO_3; 1.1 g $FeSO_4.7H_2O$; 190 mg $CoCl_2.6H_2O$; 50 mg $MnCl_2.2H_2O$; 42 mg $ZnCl_2$; 24 mg $NiCl_2.6H_2O$; 18 mg $Na_2MoO_4.2H_2O$ and 2 mg $CuCl_2$, $2H_2O$ per liter. The final concentration on sulfur compounds in the basal medium is 3.6 µM.

Cultures were performed with 40 mM pyruvate as carbon source and either with 40 mM sulfate (sulfidogenic condition) or with 40 mM fumarate (non-sulfidogenic metabolism). Under non-sulfidogenic growth, 0.1 mM cysteine, or a wide range of sulfide (Na_2S) concentration (from 0.0005 to 5 mM) was added as sulfur source. In growth conditions requiring sulfides addition, sulfides were supplemented 24 hours before the inoculation enabling equilibration with the culture medium maintained in anoxic/reduced condition. Cultures were inoculated at 0.03 optical density (OD) measured with a spectrophotometer at 600 nm, from pre-cultures under the same growth condition. Growth was then monitored by OD, and biomass production (ΔOD_{600}) was calculated as [OD (Tf) – OD (Ti)], where Tf corresponds to the end of the exponential phase and Ti to the time of the inoculation of the strain. Growth rate (µ (h^{-1})) was obtained by dividing the produced biomass during the exponential phase ($\ln(biomassproduction)$) by time of the exponential growth phase.

Cells numbering was performed by flow cytometry (BD Accuri C6 analysor, TBMCore). Aliquots of 1.6 mL of cultures were stored at -80°C in 5% (v/v) filtered formaldehyde until cells counting. Samples were tagged with 10X SYBR® (Invitrogen) for 15 minutes in the dark, and immediately counted using Trucounts beads (BD). Sulfide quantification was performed by Cline (Cline 1969) method using a spectrophotometer at 670 nm and adapted to growth conditions and sulfide concentrations expected. The detection limit was 0.92 µM.

Hg methylation assays and Hg species partitioning

All methylation assays were conducted in autoclaved Hungate tubes pre-washed by ultrasonication in 10% HNO_3 and HCl baths, and rinsed with ultrapure Milli-Q water. Inorganic Hg(II) stock solution (5 mM $HgCl_2$ in 1% HCl) was used to yield final concentrations of 0.05 µM, 2 µM or 5 µM.

In order to avoid heterogeneity, media flushed with N_2 were inoculated with BerOc1 strain and distributed in tubes containing 100 µL of Hg(II) at the appropriate concentration. The fully filled tubes were closed with PTFE coated butyl stoppers, mixed and incubated in the dark at 37°C. Inoculation and Hg addition were thus performed simultaneously (**Online Resource 1a**). Quantification of Hg species (i.e. inorganic mercury, Hg(II) and, methylmercury, MeHg), sulfide concentration and cell numbering in the bulk culture were determined at both Ti (just after inoculation and Hg addition) and Tf (at the end of the exponential growth phase). For Hg(II) and MeHg quantification (bulk fraction), an aliquot of 500 µL of the cell culture was collected and digested in 50% (v/v) of 6N nitric acid (HNO_3). The remaining part of the cultures was centrifuged (30 min, 6000 g at 4°C) to separate supernatant and pellet fractions (**Online Resource 1a**).

500 µL of the supernatant was collected and digested in 50% (v/v) 6N HNO_3 (extracellular fraction). Samples were stored at 4°C before the analyses. Abiotic controls without cells were performed under the same experimental conditions for the three different culture media.

Hg(II) and MeHg concentrations were measured by capillary gas chromatography (GC TriPlus™ RSH™, Thermo Scientific) connected to an inductively coupled plasma mass spectrometer (ICPMS, X2-series, Thermo Electron)(Bridou et al. 2011; Pedrero et al. 2012). Quantification of each Hg species concentration was performed by isotopic dilution (Monperrus et al. 2005). Briefly, a known amount of fractions (bulk or supernantant fractions) was buffered at pH 3.9 using a 0.1 M acetic acid/acetate buffer and then spiked with a known quantity of isotopically enriched Hg species (^{199}Hg(II) and ^{201}MeHg). Hg(II) and MeHg were ethylated using 5% (v/v) $NaBEt_4$ and extracted in isooctane by shaking vigorously during 20 min. Organic phase containing Hg species was collected and analyzed by GC-ICPMS. Each fraction was measured two times.

Hg methylation potential and Hg species partitioning were determined in the same batch culture. Methylation potentials were calculated in the bulk cultures by dividing MeHg concentration produced (Ti - Tf) by total Hg concentration measured at Ti. Partitioning of each Hg species (Hg(II) and, MeHg) between cell-associated and extracellular fractions was calculated by dividing concentrations of Hg species measured in the supernatant (extracellular fraction) by concentrations of Hg species measured in the bulk fraction x100. Percentage of Hg species in the pellet (i.e. cell-associated fraction (sorbed and intracellular)), were determined by subtracting the percentage of Hg species in the supernatant fraction to 100%.

Spearman's correlation between the percentage of MeHg in extracellular fraction and sulfide concentrations was calculated with R software.

hgcA **expression assays and analyses**

hgcA expression assays were performed under various Hg(II) concentration exposures in non sulfidogenic condition with either cysteine or sulfide (0.1 mM). Growth was performed as previously described except that (i) Hg(II) was added at mid-exponential growth phase in the anaerobic chamber and, (ii) Hg incubation was carried out for one hour (**Online Resource 1b**). Based on Hg methylation results, *hgcA* expression level was measured at Hg(II) concentrations of 0.05 µM, 2 µM and 5 µM with sulfide and, 0.005 µM, 0.05 µM, 0.5 µM, 2 µM with cysteine. Controls without Hg(II) were conducted to determine the basal *hgcA* expression.

After 1 hour of incubation with Hg(II), cultures were stopped with 3% (v/v) of RNA protect solution (5% phenol saturated with Na acetate 1M pH 5.5 into 95% ethanol). Five mL of the culture were centrifuged (10 min, 5000 g) and total RNA was extracted using EXTRACT-ALL® Kit (eurobio) according to the kit protocol guidelines. RNA fraction was purified by removing DNA contamination with TURBO DNase RNase-free™ Kit (Ambion) and quantified with Quant-iT RNA Assay Kit (Invitrogen™) and Synergy HTX

multi-mode reader (BioTek). Purified RNA was diluted at 20 ng/µL and cDNA was synthesized using M-MLV-RT (Invitrogen).

hgcA transcripts were quantified by qRT-PCR (quantitative Reverse-transcription PCR) in a Light Cycler 480 (Roche) using the DyNAmo™ ColorFlash SYBR® Green qPCR Kit (Finnzyme), in a final volume of 20 µL with 1 µL of cDNA and the appropriate primers(Goñi-Urriza et al. 2015). *gyrB* gene expression was used as housekeeping gene expression for normalization (Goñi-Urriza et al. 2015). *hgcA* and *gyrB* primers were specifically designed to amplified *hgcA* and *gyrB* genes in *P. hydrargyri* strain BerOc1 in a previous study(Goñi-Urriza et al. 2015). Real time PCR was carried out with an initial denaturation of 7 min at 95°C followed by 45 cycles of 10 sec at 95°C and 20 sec at 60°C. Melt characteristics of amplicons were checked at the end of the amplification. Assays were performed in three independent cultures (biological replicates) each one measured three times (technical replicates). A reaction mixture with DNA-free RNA was run as a control for detection of DNA contamination. Amplification efficiencies of *hgcA* and *gyrB* primers were determined with standard curves as previously described (Goñi-Urriza et al. 2015), and were of 1.90 and 1.95, respectively.

hgcA induction level was determined following double delta Ct (Cycle Threshold) procedure using *gyrB* expression as reference. Briefly it consists of: (i) a first normalization of the target gene expression (*hgcA*) with a reference gene expression (*gyrB*) and (ii) a second normalization of the Ct in the induced condition (growth with Hg(II)) with the Ct of the non-induced condition (growth without Hg(II)).

Results And Discussion

Hg methylation is driven by sulfides

The SRB *P. hydrargyri* strain BerOc1, able to use sulfate or fumarate as electron acceptors, was used in this study to determine the link between MeHg production and sulfide. The production of MeHg was assessed at three different Hg(II) concentrations (0.05, 2 and 5 µM of Hg(II)) under non-sulfidogenic growth (Pyruvate (Pyr)/Fumurate) with cysteine or sulfide added exogenously (0.1 mM) and under sulfidogenic growth (Pyr/Sulfate). The chosen cysteine and sulfide concentrations correspond to the minimal sulfur concentration needed for optimal growth without Hg(II) (**Online Resource 2a**). The Hg(II) concentrations were chosen from a previous study that showed a maximum of mercury methylation rate at 5 µM of Hg(II) (Isaure et al. 2020). Sulfide concentrations measured at the end of the growth in the presence of cysteine (0.010 ± 0.003 - 0.020 ± 0.005 mM, **Table 1**) are consistent with previous sulfide concentrations reported for degradation of cysteine by bacteria, including the well-known Hg methylators *Geobacter sulfurreducens* PCA and *Pseudodesulfovibrio mercurii* ND132 (Graham et al. 2012a, b; Thomas and Gaillard 2017; Thomas et al. 2018). Moreover, for a given growth condition, addition of Hg(II) did not impact neither bacterial growth nor sulfide concentration (**Table 1, Online Resource 2d-f**).

Interestingly, the Hg(II) methylation potential under non-sulfidogenic growth with 0.1 mM sulfide was higher than in the other tested conditions, regardless of the Hg(II) concentration (Figure 1a, **Online**

Resource Table). While a progressive decrease of Hg methylation potential over an increase of Hg(II) concentrations was detected under sulfidogenic growth, a drastic decline over 0.05 µM was noticed under non-sulfidogenic growth with cysteine (from 15% to < 1%) (Figure 1a). However, under non-sulfidogenic growth with sulfide only a moderate decline was observed above 2 µM Hg(II). Furthermore, the maximal rate of production of MeHg per cell (τ_{max} calculation) is between 50 and 100 fold higher (**Table 1**) in presence of sulfide. The saturation of MeHg production (from $K_{Michaelis}$ calculation) of BerOc1 grown with sulfide is reached at higher Hg(II) concentrations than when grown with sulfate (10 times lower than sulfide) or cysteine (1000 times lower than sulfide). These results indicated that the Hg methylation mechanism is saturable in the various cell growth conditions tested as previously described under a different growth condition than tested here (Isaure et al. 2020). Abiotic controls confirmed that measured Hg methylation was a fully biotic process and that the observed differences between growth conditions were not related to differences in abiotic Hg methylation (**Online Resource 3**). Furthermore, our study reported for the first time that the concentration at which this saturation is reached and the capacity of a single cell to produce MeHg depends on growth conditions.

Importantly, Hg methylation potentials were higher with 0.1 mM of sulfide, compared to cysteine condition (expected to favor Hg methylation (Schaefer and Morel 2009)) regardless of the Hg(II) concentration added (Figure 1a). However, in presence of cysteine around 10 µM of sulfides were measured (**Table 1**). We thus hypothesized that the variation observed in Hg methylation depends on sulfide concentration (added or produced) rather than directly on the added cysteine. Therefore, we investigated Hg(II) methylation at varying concentration of sulfides (from 0.0005 to 5 mM). The range of sulfide concentrations were chosen following the measured sulfide production under cysteine and sulfate reduction growth (**Table 1**). Two Hg(II) concentrations, 0.05 and 2 µM Hg(II) (where the differences between Hg(II) potential are the most prominent (Figure 1a)), were used to test the effect of sulfide concentration on Hg(II) methylation potentials. Addition of low exogenous sulfide concentrations (< 0.05 mM) limited BerOc1 growth only at 2 µM of Hg (II) while sulfide above 2 mM was toxic for the growth, regardless of the Hg(II) concentration added (**Table 1, Online Resource 2b-c**). At 0.05 µM of Hg(II), Hg methylation potentials increased with the increase of sulfide concentrations up to 0.5 mM, and then decreased at higher sulfide concentrations (Figure 1b). The same pattern was observed with 2 µM of Hg(II) with a maximal Hg methylation reached at 0.1 mM of sulfides (Figure 1c). Overall, our data showed that sulfide at concentration ranged from 0.1 to 0.5 mM favors Hg methylation in *P. hydrargyri* BerOc1 and that lower sulfide concentration (< 0.1 mM) and higher sulfide concentration (> 0.5 mM) from that range are disadvantageous to the process. Several previous studies concluded that the presence of sulfide has an inhibitory effect on Hg methylation (Benoit et al. 1999a, a). The inhibitory effect on Hg methylation at high sulfide concentration was for instance observed in *Desulfobulbus propionicus* (1pr3) by testing sulfide concentration from 0.05 to 2 mM (Benoit et al. 2001a). However, because only one concentration of sulfide below 0.1 mM was tested in this study, the beneficial effect of sulfide on Hg methylation was not noticed on the plot. Additionally, an optimum of sulfide concentration (between 0.01 and 0.1 mM) favoring Hg methylation has been observed in methanogens (Gilmour et al. 2018). The modeling linking sulfate concentration to MeHg production in natural ecosystems also identified a similar

pattern, with an optimum of Hg methylation ranging from 0.2 to 0.5 mM of sulfate (Gilmour and Henry 1991). Since the optimal concentration of sulfide favoring Hg methylation in our study is close to the optimal concentration of sulfate favoring Hg methylation in Gilmour and Henry study (Gilmour and Henry 1991), we can suggest that sulfide is the main parameter here favoring Hg methylation, since sulfate can be reduced to sulfide.

The mechanism by which S containing molecules favors Hg methylation is far from being understood. Thomas *et al* (Thomas et al. 2020), identified Hg-S complexes in non-growing *Geobacter sulfurreducens* PCA (a Hg(II) methylating bacterium) incubated with cysteine. In this study, the addition of cysteine also increased Hg(II) methylation and the authors suggested that the degradation of cysteine produces sulfide that forms Hg-S complexes and those complexes would favor Hg(II) methylation through a facilitated Hg uptake, as demonstrated in *E. coli* (Thomas et al. 2019). Unfortunately, sulfide production by *G. sulfurreducens* PCA was not provided. Further spectroscopic analysis and characterization of Hg-S complexes under a range of sulfide concentrations would shed light on the role of sulfide at 0.1-0.5 mM in the production of Hg-S forms and the increase of Hg(II) methylation.

Overall, how sulfide, sulfate (Gilmour and Henry 1991) and cysteine (Schaefer and Morel 2009) affect Hg methylation depends mainly on their concentrations. In marine environments, sulfate can be found at concentrations up to 20 mM (Jørgensen et al. 2019) and cysteine at fM to nM concentrations (Joe-Wong et al. 2012; Liem-Nguyen et al. 2017; Bouchet et al. 2018; Adediran et al. 2019). At those concentrations, sulfate and cysteine are not expected to play a major role in MeHg production (Gilmour and Henry 1991; Schaefer and Morel 2009). However, other S-containing molecules, especially those involved in the sulfur biogeochemical cycle (sulfide, sulfite, thiosulfate, and polysulfide), reach concentrations between 0.1 and 1 mM in marine and freshwater sediments (Findlay 2016; Findlay and Kamyshny 2017; Gentès et al. 2017) and can all be transformed into sulfide by reduction or degradation. Here, we reported an increase of Hg methylation at sulfide concentration encountered in the environment. We hypothesize that in natural environments, sulfate will enhance Hg methylation because it favors SRB metabolism but sulfide (regardless of its origin, exogenous or endogenous) will govern the extent of Hg methylation potentials.

Sulfides influence methylmercury partitioning

In a previous study, a link between Hg species partitioning and Hg methylation was observed with various thiols molecules (Schaefer and Morel 2009). Thus, to further understand differences on Hg methylation observed with various S containing molecules and sulfide concentration in our study, we investigated the Hg species (Hg(II) and MeHg) partitioning between the extracellular and the cell-associated (cell-sorbed and intracellular) fractions in BerOc1 grown under the three growth conditions (Pyr/Fumarate with cysteine or sulfide and, Pyr/Sulfate) and with sulfide gradient under Pyr/Fumarate condition. Distribution of Hg species was measured at the beginning of the growth (Ti, Hg(II), **Online Resource 4**) and at the end of exponential phase (Tf, Hg(II) and MeHg produced) at increasing Hg(II) concentrations (Figure 2, Figure 3). For a given growth condition, increasing Hg(II) concentrations did not affect neither Hg(II) (Figure 2a) nor MeHg (Figure 3a) partitioning. Hg(II) became more associated with the cells over time (**Online Resource 4a**, Figure 3a) and, was mostly associated with cells at the end of the exponential growth phase

(80-100%) regardless of the growth condition (Figure 3a). On the contrary, MeHg distribution between extracellular and the cell fractions depends on the growth condition (Figure 3a): while most of the MeHg was detected in the extracellular fraction under sulfidogenic growth (96 ± 2%), the proportion in the extracellular fraction reached only 30 ± 4% under non-sulfidogenic condition with cysteine. An intermediate distribution (50 ± 5%) of MeHg was observed in non-sulfidogenic growth with sulfide. Thus, the proportion of extracellular MeHg increased with the increasing sulfide concentrations measured in the cultures (Figure 3a, **Table 1**). The dominance of produced MeHg in the extracellular medium has been already observed and was interpreted as an export of MeHg by the bacteria or desorption from the cells (Graham et al. 2012b; Liu et al. 2016; Pedrero et al. 2012). We thus infer that the export/desorption of MeHg from the bacterial cells can be favored by sulfide.

In order to test this hypothesis, we measured the distribution of Hg species in the extracellular and the cell-associated fractions in BerOc1 cells grown with increasing exogenous sulfide concentrations. At both 0.05 and 2 µM of Hg(II), Hg(II) became more associated to the cell fraction over time for all of the sulfide concentrations tested (**Online Resource 4b-c and** Figure 2b-c), as observed for BerOc1 under Pyr/Fumarate with cysteine and Pyr/Sulfate conditions (**Online Resource 4a and** Figure 2a). Thus, Hg(II) partitioning appear not to be influenced by sulfide concentration. Remarkably, the extracellular MeHg increased with the increase of sulfide concentrations regardless of the Hg(II) concentration added (Figure 3b, Figure 3c). A strong positive correlation was observed (Spearman correlation rho = 0.89, p value < 0.001). Previous studies showed an increase of the extracellular MeHg in presence of thiols (Ndu et al. 2016). More specifically, cysteine strongly enhanced the extracellular MeHg produced by *G. sulfurreducens* PCA and more slightly by *Pseudodesulfovibrio mercurii* ND132. Accordingly, a facilitated MeHg cell export and desorption from the cells was proposed via the cysteine (Lin et al. 2015). However, no data of sulfide contents was provided (Lin et al. 2015) and, in the light of the recent papers (Thomas et al. 2019), it may be possible that the facilitated export/desorption was the consequence of sulfide production due to cysteine degradation. Our results show that sulfide, either exogenous or endogenous (produced by the cell via cysteine degradation or sulfate reduction), could be a strong parameter involved in MeHg export and/or desorption from the cell.

A recent study on abiotic interaction of MeHg and sulfide predicted the formation of bismethylmercury sulfide as the dominant MeHg species in sulfidic solutions (Kanzler et al. 2018). In mammalian cells, the bismethylmercury sulfide formation from MeHg has been proposed as a mechanism of cell detoxification (Yoshida et al. 2011). In this study, sulfide (exogenous or produced via cysteine and homocysteine degradation) form bismethylmercury sulfide in the presence of MeHg, avoiding MeHg binding to cellular proteins (Yoshida et al. 2011). In our study, the progressive decrease of cell associated MeHg observed when sulfide concentration increased could be explained by the formation of bismethylmercury sulfide that avoid the binding of MeHg to cellular proteins. However, it is difficult to speculate on the combined role of Hg methylation and MeHg export for Hg cell detoxification since no link between both Hg methylation potentials and MeHg export could be found. MeHg export probably protects BerOc1 cells against MeHg toxicity. In the other hand, the decrease of Hg methylation potential observed at high

sulfide concentrations may be the consequence of a lower availability of Hg because of Hg-S complexes, limiting Hg toxicity.

The increase of Hg methylation in presence of sulfide is not associated to hgcA **overexpression**

hgcAB are the only genetic determinisms identified as necessary for Hg methylation(Parks et al. 2013). To investigate if the increase of Hg methylation observed with 0.1 mM of sulfide was related to the overexpression of the enzymatic mechanism of Hg methylation, the expression of *hgcA* gene (involved in the methyl transfer to Hg) was measured in BerOc1. We selected cells growing under non-sulfidogenic growth with 0.1 mM sulfide and 0.1 mM cysteine, where Hg methylation was lower (Figure 1a). Low *hgcA* overexpression (from 1.8 to 2.2) was observed for cysteine for all the Hg(II) concentrations tested (Figure 4a). In contrast, no overexpression could be detected for 0.1 mM sulfide, where the methylation potentials were higher (Figure 1a **and** Figure 4b). Thus, no direct link could be established between *hgcA* expression and Hg methylation potentials. The high Hg methylation potential observed is likely linked to the cellular environment, such as sulfides reported here or substrates used for bacterial growth as previously reported (Goñi-Urriza et al. 2015). In addition, our results revealed that *hgcA* gene is expressed at basal level even at increasing Hg(II) concentrations. Hg(II) seems not control *hgcA* expression, unlike other metals (Zn, Cu, As, Fe) which regulate the expression of genes encoding enzymes controlling their transformation (Andrei et al. 2020; Andrews et al. 2003; Pederick et al. 2015; Silver 1996; Silver and Phung 2005). Nevertheless, the available data regarding the expression of *hgcAB* gene are so far insufficient to ascertain that *hgcAB* genes are not regulated. A transcriptional regulator, *arsR*, located upstream *hgcAB* genes and cotranscribed with *hgcA* clearly suggests a regulated process (Goñi-Urriza et al. 2020). Its implication in the regulation of *hgcAB* genes expression and the factors that trigger the ArsR response are still unknown and should be addressed in future studies.

Conclusion

In this work, the influence of sulfide regardless of its origin (added exogenously or produced endogenously from cysteine or sulfate) on mercury methylation and Hg species partitioning was investigated using the model strain *Pseudodesulfovibrio hydrargyri* BerOc1. The mercury methylation assays showed that there is a range of sulfide concentration (0.1 to 0.5 mM) that enhance mercury methylation. Remarkably, no matter the S containing molecules used, the driven parameter is the sulfide concentration, whatever if the molecule is added exogenously or produced by the cells (via cysteine degradation or sulfate reduction).

Importantly, partitioning assays also demonstrated that MeHg export increases with increasing sulfide concentration. A drastic shift was observed: low sulfide concentrations lead to MeHg being associated to the cells while high sulfide concentrations results in extracellular MeHg, likely exported from the cells. Despite the lack of correlation between MeHg export and Hg methylation in this study, such a shift in partitioning of MeHg, was never observed. Sulfide has a central role in determining MeHg production and export. The evaluation of the concentration of sulfide in natural environments is thus important and

should provide information on the possibility of the neurotoxic methylmercury spread in the aquatic environment, in order to better evaluate the risks of environmental Hg contamination and its impact on public health.

Declarations

Ethical Approval

All authors declare no violation of the ethical rules of ESPR Journal

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Competing interests

The authors declare no competing interests.

Authors consent and contributions

All authors consented to participate and publish this work.

Contributions: S.B.: Design of the work, acquisition, analysis and interpretation of data, writing original draft. M.M.: Design of the work, data curation, review & editing. E.T.: Data curation, methodology. B.K-H: review & editing. R.G.: Design of the work, analysis, methodology, review & editing. M-P.I.: Design of the work, funding acquisition, methodology, project administration, supervision, writing original draft, review & editing. M.G-U.: Design of the work, analysis, methodology, supervision, writing original draft, review & editing.

Availability of data and materials

Data will be available upon request

Supplementary Information

Additional details about experimental procedures, cell growth and experimental controls are found in SI. Online Resource 1 is a schematic representation of the experimental key times. Online Resource 2 shows the growth curves of cultures conducted in this study. Online Resource 3 shows growth curves, Hg methylation potential and total recovered Hg measured in abiotic control (without cells) and Online Resource 4 shows initial partitioning of inorganic mercury (sampled at Ti). Online Resource Table shows the raw data of Hg methylation assays.

References

1. Adediran GA, Liem-Nguyen V, Song Y, et al (2019) Microbial Biosynthesis of Thiol Compounds: Implications for Speciation, Cellular Uptake, and Methylation of Hg(II). Environ Sci Technol 53:8187–8196. https://doi.org/10.1021/acs.est.9b01502
2. Andrei A, Öztürk Y, Khalfaoui-Hassani B, et al (2020) Cu Homeostasis in Bacteria: The Ins and Outs. Membranes 10:242. https://doi.org/10.3390/membranes10090242
3. Andrews SC, Robinson AK, Rodríguez-Quiñones F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27:215–237. https://doi.org/10.1016/S0168-6445(03)00055-X
4. Atchison WD, Hare MF (1994) Mechanisms of methylmercury-induced neurotoxicity. FASEB J. 8:622–629. https://doi.org/10.1096/fasebj.8.9.7516300
5. Benoit JM, Gilmour CC, Mason RP, Heyes A (1999a) Sulfide Controls on Mercury Speciation and Bioavailability to Methylating Bacteria in Sediment Pore Waters. Environ Sci Technol 33:951–957. https://doi.org/10.1021/es9808200
6. Benoit JM, Mason RP, Gilmour CC (1999b) Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and implications for availability to methylating bacteria: Mercury-sulfide speciation. Environ Toxicol Chem 18:2138–2141. https://doi.org/10.1002/etc.5620181004
7. Benoit JM, Gilmour CC, Mason RP (2001a) The Influence of Sulfide on Solid-Phase Mercury Bioavailability for Methylation by Pure Cultures of *Desulfobulbus propionicus* (1pr3). Environ Sci Technol 35:127–132. https://doi.org/10.1021/es001415n
8. Benoit JM, Gilmour CC, Mason RP (2001b) Aspects of Bioavailability of Mercury for Methylation in Pure Cultures of Desulfobulbus propionicus (1pr3). Appl Environ Microbiol 67:51–58. https://doi.org/10.1128/AEM.67.1.51-58.2001
9. Bouchet S, Goñi-Urriza M, Monperrus M, et al (2018) Linking Microbial Activities and Low-Molecular-Weight Thiols to Hg Methylation in Biofilms and Periphyton from High-Altitude Tropical Lakes in the Bolivian Altiplano. Environ Sci Technol 52:9758–9767. https://doi.org/10.1021/acs.est.8b01885
10. Boudou A, Ribeyre F (1997) Mercury in the food web: accumulation and transfer mechanisms. Met Ions Biol Syst 34:289–319
11. Bravo AG, Zopfi J, Buck M, et al (2018) Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases. ISME J. 12:802–812. https://doi.org/10.1038/s41396-017-0007-7
12. Bridou R, Monperrus M, Gonzalez PR, et al (2011) Simultaneous determination of mercury methylation and demethylation capacities of various sulfate-reducing bacteria using species-specific isotopic tracers. Environ Toxicol Chem 30:337–344. https://doi.org/10.1002/etc.395
13. Cline JD (1969) SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN SULFIDE IN NATURAL WATERS1. Limnol Oceanogr 14:454–458. https://doi.org/10.4319/lo.1969.14.3.0454
14. Compeau GC, Bartha R Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sedimentt. APPL ENVIRON MICROBIOL 5

15. Drott A, Lambertsson L, Björn E, Skyllberg U (2007) Importance of Dissolved Neutral Mercury Sulfides for Methyl Mercury Production in Contaminated Sediments. Environ Sci Technol 41:2270–2276. https://doi.org/10.1021/es061724z
16. Farina M, Rocha JBT, Aschner M (2011) Mechanisms of methylmercury-induced neurotoxicity: Evidence from experimental studies. Life Sci. 89:555–563. https://doi.org/10.1016/j.lfs.2011.05.019
17. Findlay AJ (2016) Microbial impact on polysulfide dynamics in the environment. FEMS Microbiol Let 363:fnw103. https://doi.org/10.1093/femsle/fnw103
18. Findlay AJ, Kamyshny A (2017) Turnover Rates of Intermediate Sulfur Species (Sx2-, S0, S2O32-, S4O62-, SO32-) in Anoxic Freshwater and Sediments. Front Microbiol 8:. https://doi.org/10.3389/fmicb.2017.02551
19. Gentès S, Taupiac J, Colin Y, et al (2017) Bacterial periphytic communities related to mercury methylation within aquatic plant roots from a temperate freshwater lake (South-Western France). Environ Sci Pollut Res 24:19223–19233. https://doi.org/10.1007/s11356-017-9597-x
20. Gilmour CC, Henry EA (1991) Mercury methylation in aquatic systems affected by acid deposition. Environ Pollut 71:131–169. https://doi.org/10.1016/0269-7491(91)90031-Q
21. Gilmour CC, Elias DA, Kucken AM, et al (2011) Sulfate-Reducing Bacterium Desulfovibrio desulfuricans ND132 as a Model for Understanding Bacterial Mercury Methylation. Appl Environ Microbiol 77:3938–3951. https://doi.org/10.1128/AEM.02993-10
22. Gilmour CC, Podar M, Bullock AL, et al (2013) Mercury Methylation by Novel Microorganisms from New Environments. Environ Sci Technol 47:11810–11820. https://doi.org/10.1021/es403075t
23. Gilmour CC, Bullock AL, McBurney A, et al (2018) Robust Mercury Methylation across Diverse Methanogenic Archaea. mBio 9:e02403-17. https://doi.org/10.1128/mBio.02403-17
24. Goñi-Urriza M, Corsellis Y, Lanceleur L, et al (2015) Relationships between bacterial energetic metabolism, mercury methylation potential, and hgcA/hgcB gene expression in Desulfovibrio dechloroacetivorans BerOc1. Environ Sci Pollut Res 22:13764–13771. https://doi.org/10.1007/s11356-015-4273-5
25. Goñi-Urriza M, Klopp C, Ranchou-Peyruse M, et al (2020) Genome insights of mercury methylation among Desulfovibrio and Pseudodesulfovibrio strains. Res Microbiol 171:3–12. https://doi.org/10.1016/j.resmic.2019.10.003
26. Graham AM, Aiken GR, Gilmour CC (2012a) Dissolved Organic Matter Enhances Microbial Mercury Methylation Under Sulfidic Conditions. Environ Sci Technol 46:2715–2723. https://doi.org/10.1021/es203658f
27. Graham AM, Bullock AL, Maizel AC, et al (2012b) Detailed Assessment of the Kinetics of Hg-Cell Association, Hg Methylation, and Methylmercury Degradation in Several Desulfovibrio Species. Appl Environ Microbiol 78:7337–7346. https://doi.org/10.1128/AEM.01792-12
28. Isaure M-P, Albertelli M, Kieffer I, et al (2020) Relationship Between Hg Speciation and Hg Methylation/Demethylation Processes in the Sulfate-Reducing Bacterium Pseudodesulfovibrio

hydrargyri: Evidences From HERFD-XANES and Nano-XRF. Front Microbiol 11:584715. https://doi.org/10.3389/fmicb.2020.584715

29. Jensen S, Jernelöv A (1969) Biological Methylation of Mercury in Aquatic Organisms. Nature 223:753–754. https://doi.org/10.1038/223753a0
30. Joe-Wong C, Shoenfelt E, Hauser EJ, et al (2012) Estimation of Reactive Thiol Concentrations in Dissolved Organic Matter and Bacterial Cell Membranes in Aquatic Systems. Environ Sci Technol 120831120231009. https://doi.org/10.1021/es301381n
31. Jørgensen BB, Findlay AJ, Pellerin A (2019) The Biogeochemical Sulfur Cycle of Marine Sediments. Front Microbiol 10:. https://doi.org/10.3389/fmicb.2019.00849
32. Kanzler CR, Lian P, Trainer EL, et al (2018) Emerging investigator series: methylmercury speciation and dimethylmercury production in sulfidic solutions. Environ Sci: Process Impacts 20:. https://doi.org/10.1039/C7EM00533D
33. Kerin EJ, Gilmour CC, Roden E, et al (2006) Mercury Methylation by Dissimilatory Iron-Reducing Bacteria. Appl Environ Microbiol 72:7919–7921. https://doi.org/10.1128/AEM.01602-06
34. Liem-Nguyen V, Skyllberg U, Björn E (2017) Thermodynamic Modeling of the Solubility and Chemical Speciation of Mercury and Methylmercury Driven by Organic Thiols and Micromolar Sulfide Concentrations in Boreal Wetland Soils. Environ Sci Technol 51:3678–3686. https://doi.org/10.1021/acs.est.6b04622
35. Lin H, Lu X, Liang L, Gu B (2015) Thiol-Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria. Environ Sci Technol Lett 2:292–296. https://doi.org/10.1021/acs.estlett.5b00209
36. Liu Y-R, Lu X, Zhao L, et al (2016) Effects of Cellular Sorption on Mercury Bioavailability and Methylmercury Production by *Desulfovibrio desulfuricans* ND132. Environ Sci Technol 50:13335–13341. https://doi.org/10.1021/acs.est.6b04041
37. Monperrus M, Tessier E, Veschambre S, et al (2005) Simultaneous speciation of mercury and butyltin compounds in natural waters and snow by propylation and species-specific isotope dilution mass spectrometry analysis. Anal Bioanal Chem 381:854–862. https://doi.org/10.1007/s00216-004-2973-7
38. Ndu U, Barkay T, Schartup AT, et al (2016) The effect of aqueous speciation and cellular ligand binding on the biotransformation and bioavailability of methylmercury in mercury-resistant bacteria. Biodegrad 27:29–36. https://doi.org/10.1007/s10532-015-9752-3
39. Overmann Jg, Fischer U, Pfennig N A new purple sulfur bacterium from saline littoral sediments, Arch Microbiol (1992) 157:329-335
40. Parks JM, Johs A, Podar M, et al (2013) The Genetic Basis for Bacterial Mercury Methylation. Sci 339:1332–1335. https://doi.org/10.1126/science.1230667
41. Pederick VG, Eijkelkamp BA, Begg SL, et al (2015) ZnuA and zinc homeostasis in Pseudomonas aeruginosa. Sci Rep 5:13139. https://doi.org/10.1038/srep13139

42. Pedrero Z, Bridou R, Mounicou S, et al (2012) Transformation, Localization, and Biomolecular Binding of Hg Species at Subcellular Level in Methylating and Nonmethylating Sulfate-Reducing Bacteria. Environ Sci Technol 46:11744–11751. https://doi.org/10.1021/es302412q
43. Qian C, Chen H, Johs A, et al (2018) Quantitative Proteomic Analysis of Biological Processes and Responses of the Bacterium *Desulfovibrio desulfuricans* ND132 upon Deletion of Its Mercury Methylation Genes. PROTEOM 18:1700479. https://doi.org/10.1002/pmic.201700479
44. Ranchou-Peyruse A, Moppert X, Hourcade E, et al (2004) Characterization of brackish anaerobic bacteria involved in hydrocarbon degradation: A combination of molecular and culture-based approaches. Ophelia 58:255–262. https://doi.org/10.1080/00785236.2004.10410233
45. Ranchou-Peyruse M, Monperrus M, Bridou R, et al (2009) Overview of Mercury Methylation Capacities among Anaerobic Bacteria Including Representatives of the Sulphate-Reducers: Implications for Environmental Studies. Geomicrobiol J 26:1–8. https://doi.org/10.1080/01490450802599227
46. Ranchou-Peyruse M, Goñi-Urriza M, Guignard M, et al (2018) Pseudodesulfovibrio hydrargyri sp. nov., a mercury-methylating bacterium isolated from a brackish sediment. Int J System Evol Microbiol 68:1461–1466. https://doi.org/10.1099/ijsem.0.002173
47. Rice KM, Walker EM, Wu M, et al (2014) Environmental Mercury and Its Toxic Effects. J Prev Med Public Health 47:74–83. https://doi.org/10.3961/jpmph.2014.47.2.74
48. Schaefer JK, Morel FMM (2009) High methylation rates of mercury bound to cysteine by Geobacter sulfurreducens. Nature Geosci 2:123–126. https://doi.org/10.1038/ngeo412
49. Silver S (1996) Bacterial resistances to toxic metal ions - a review. Gene 179:9–19. https://doi.org/10.1016/S0378-1119(96)00323-X
50. Silver S, Phung LT (2005) Genes and Enzymes Involved in Bacterial Oxidation and Reduction of Inorganic Arsenic. Appl Environ Microbiol 71:599–608. https://doi.org/10.1128/AEM.71.2.599-608.2005
51. Thomas SA, Gaillard J-F (2017) Cysteine Addition Promotes Sulfide Production and 4-Fold Hg(II)–S Coordination in Actively Metabolizing *Escherichia coli*. Environ Sci Technol 51:4642–4651. https://doi.org/10.1021/acs.est.6b06400
52. Thomas SA, Rodby KE, Roth EW, et al (2018) Spectroscopic and Microscopic Evidence of Biomediated HgS Species Formation from Hg(II)–Cysteine Complexes: Implications for Hg(II) Bioavailability. Environ Sci Technol 52:10030–10039. https://doi.org/10.1021/acs.est.8b01305
53. Thomas SA, Catty P, Hazemann J-L, et al (2019) The role of cysteine and sulfide in the interplay between microbial Hg(ii) uptake and sulfur metabolism. Metallomics 11:1219–1229. https://doi.org/10.1039/c9mt00077a
54. Thomas SA, Mishra B, Myneni SCB (2020) Cellular Mercury Coordination Environment, and Not Cell Surface Ligands, Influence Bacterial Methylmercury Production. Environ Sci Technol 54:3960–3968. https://doi.org/10.1021/acs.est.9b05915

55. Trüper HG, Pfennig N (1992) The Family Chlorobiaceae. In: Balows A, Trüper HG, Dworkin M, et al. (eds) The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. Springer, New York, NY, pp 3583–3592
56. Widdel F, Bak F (1992) Gram-Negative Mesophilic Sulfate-Reducing Bacteria. In: Balows A, Trüper HG, Dworkin M, et al. (eds) The Prokaryotes. Springer New York, New York, NY, pp 3352–3378
57. Yoshida E, Toyama T, Shinkai Y, et al (2011) Detoxification of Methylmercury by Hydrogen Sulfide-Producing Enzyme in Mammalian Cells. Chem Res Toxicol 24:1633–1635. https://doi.org/10.1021/tx200394g

Tables

Table 1 Sulfide concentration, biomass production, growth rate, Hg methylation rate (τ), maximal Hg methylation rate (τ_{max}) and, Hg(II) concentrations to reach half of the maximal methylation rates (K_{Mic}) measured/calculated in *P. hydrargyri* strain BerOc1 grown under non-sulfidogenic (Pyr/Fum) conditions (with cysteine or sulfide) or under sulfidogenic (Pyr/Sulfate) condition

Growth condition	Total added Hg (µM)	Exogenously added sulfide (mM)	Total measured sulfide [a] (mM)	Biomass production [a] (ΔOD_{600})	Growth rate (h^{-1})	Hg methylation rate $*10^{-21}$ ($mol.cell^{-1}.h^{-1}$) τ [b]	τ_{max} $*10^{-21}$ [c] ($mol.cell^{-1}.h^{-1}$)	K_{Mic} [d] (µM)
Pyr/Fum + Cysteine 0.1 mM (Non sulfidogenic)	0	0	0.02 ± 0.005	0.35 ± 0.014	0.05 ± 0.002	-	1.11	0.1
	0.05	0	0.01 ± 0.003	0.35 ± 0.014	0.05 ± 0.000	0.41 ± 0.044		
	2	0	0.01 ± 0.002	0.31 ± 0.011	0.04 ± 0.001	0.96 ± 0.119		
	5	0	0.02 ± 0	0.31 ± 0.013	0.04 ± 0.003	1.16 ± 0.019		
Pyr/Fum + Sulfide 0.1 mM (Non sulfidogenic)	0	0.1	0.11 ± 0.018	0.47 ± 0.014	0.05 ± 0.003	-	111.11	89
	0.05	0.1	0.08 ± 0.008	0.47 ± 0.030	0.05 ± 0.003	0.06 ± 0.009		
	2	0.1	0.10 ± 0.002	0.48 ± 0.016	0.05 ± 0.003	2.93 ± 0.109		
	5	0.1	0.09 ± 0.013	0.44 ± 0.002	0.04 ± 0.005	4.48 ± 0.204		
Pyr/Sulfate (Sulfidogenic)	0	0	5.15 ± 0.563	0.44 ± 0.019	0.12 ± 0.002	-	2.00	1
	0.05	0	5.49 ± 0.817	0.45 ± 0.022	0.12 ± 0.002	0.09 ± 0.005		
	2	0	5.53 ± 0.081	0.45 ± 0.016	0.12 ± 0.004	1.33 ± 0.062		
	5	0	5.34 ± 0.445	0.43 ± 0.014	0.11 ± 0.001	1.45 ± 0.040		
Pyr/Fum + Sulfide (Range of sulfide)	0.05	0.0005	0.0015 ± 0.0003	0.05 ± 0.003	0.02 ± 0.000	0.46 ± 0.049	-	-
	0.05	0.005	0.002 ± 0.0005	0.06 ± 0.006	0.03 ± 0.003	1.16 ± 0.144		
	0.05	0.05	0.012 ± 0.002	0.25 ± 0.042	0.04 ± 0.007	2.17 ± 0.213		
	0.05	0.5	0.50 ± 0.051	0.51 ± 0.067	0.04 ± 0.004	0.27 ± 0.040		
	0.05	2.4	3.19 ± 0.050	0.70 ± 0.012	0.06 ± 0.001	0.06 ± 0.003		
	0.05	4.9	10.05 ± 0.598	0	0	0		
	2	0.0005	< LD	0	0	0	-	-
	2	0.005	< LD	0	0	0		
	2	0.05	0.011 ± 0.0015	0.23 ± 0.046	0.04 ± 0.010	13.18 ± 1.302		
	2	0.5	0.47 ± 0.054	0.52 ± 0.088	0.04 ± 0.004	5.98 ± 0.767		
	2	2.4	3.02 ± 0.291	0.72 ± 0.027	0.03 ± 0.005	1.08 ± 0.027		
	2	4.9	9.67 ± 0	0	0	0		

[a] Sulfide concentration in cultures was measured at the end of the exponential growth phase (Tf). Limit of Detection (LD). Biomass production (ΔOD600) was determined by subtracting optical density measured at the end of the exponential growth phase (Tf) from OD measured at the time of strain inoculation (Ti). Standard deviations were calculated from at least three independent replicates.

[b] : Hg(II) Methylation rates (τ) $\tau = \frac{([MeHg_{Tf}] - [MeHg_{Ti}])}{\mathcal{C}_{Tf} * \text{Incubation time}}$ where the MeHg concentration produced (Tf - Ti) was divided by (concentration of cells produced during growth (Tf-Ti)) and the incubation time with Hg(II).

[c] τ_{max} : Maximal Hg methylation rate was calculated based on a Double-Reciprocal or *Lineweaver-Burk* Plot $\frac{1}{\tau} = f(\frac{1}{Hg(II)\ concentration})$

[d] K_{Mic} : Michaelis-Menten constant represents the Hg(II) concentration to reach half of the maximal methylation rate determined by the *Lineweaver-Burk* Plot.

Figures

Figure 1

Hg methylation potential (%) of P. hydrargyri BerOc1. (a) Hg methylation at 0.05, 2 and 5 µM Hg(II) under non-sulfidogenic growth (Pyr/Fum) with either 0.1 mM cysteine or 0.1 mM sulfide or under sulfidogenic condition (Pyr/Sulfate). Hg methylation at either (b) 0.05 µM or (c) 2 µM of Hg(II) under Pyr/Fum with a range of sulfide concentrations (white circle: added exogenously; gray triangle: measured in the cysteine growth condition; gray diamond: measured in the sulfidogenic growth condition). Inoculation and Hg(II) addition were performed at the same time. Pyruvate (Pyr) was used as carbon source at 40 mM for all the growth conditions and, sulfate and fumarate (Fum) were added at 40 mM as electron acceptor to promote sulfidogenic (Pyr/Sulfate) or non-sulfidogenic (Pyr/Fum) metabolisms, respectively. MeHg potentials (% of total recovered Hg converted to MeHg) were measured at the end of the exponential phase (Tf). Error bars represent the standard deviations of three independent replicates. NG: No growth

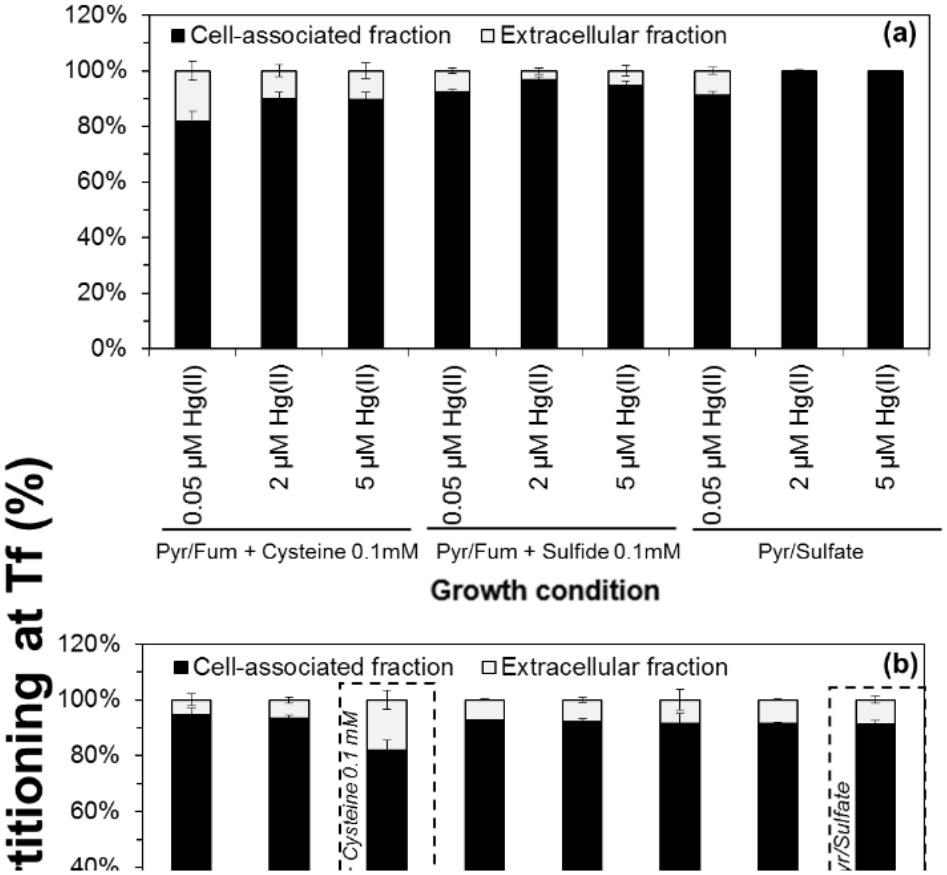

Figure 2

Partitioning of inorganic mercury (Hg(II)) between extracellular (grey) and cell-associated (black) fractions at the end of the exponential growth phase (Tf). (a) The partitioning of Hg(II) at 0.05, 2 and 5 µM Hg(II) under non-sulfidogenic growth (Pyr/Fum) with either 0.1 mM cysteine or 0.1 mM sulfide or under sulfidogenic condition (Pyr/Sulfate). The partitioning of Hg(II) at either (b) 0.05 µM or (c) 2 µM of Hg(II) under Pyr/Fum with a range of sulfide concentrations (added as exogenous sulfide or measured in

the cultures). The same cultures were used to determine Hg species in the bulk fraction at the end of the exponential growth phase and Hg species partitioning. After sampling for Hg species determination, cultures were centrifuged and the extracellular Hg(II) and MeHg were measured in the supernatant. Error bars represent the standard deviations of three independent replicates, each one measured three times

Figure 3

Partitioning of MeHg between extracellular (grey) and cell-associated (black) fractions at the end of the exponential growth phase (Tf). (a) The partitioning of MeHg at 0.05, 2 and 5 µM Hg(II) under non-sulfidogenic growth (Pyr/Fum) with either 0.1 mM cysteine or 0.1 mM sulfide or under sulfidogenic condition (Pyr/Sulfate). The partitioning of MeHg at either (b) 0.05 µM or (c) 2 µM of Hg(II) under Pyr/Fum with a range of sulfide concentrations (added as exogenous sulfide or measured in the cultures). The same cultures were used to determine Hg species in the bulk fraction at the end of the exponential growth phase and Hg species partitioning. After sampling for Hg species determination, cultures were centrifuged and the extracellular Hg(II) and MeHg were measured in the supernatant fraction. Error bars represent the standard deviations of three independent replicates, each one measured three times

Figure 4

Expression level of hgcA gene in P. hydrargyri strain BerOc1 under non-sulfidogenic growth (Pyr/Fum) with cysteine (a) and sulfide (b) at various Hg(II) concentrations. Hg(II) was spiked at the beginning of the exponential growth phase and incubated for 1 hour. A control condition was performed without Hg(II) (0 µM). Expression levels of hgcA gene were normalized using gyrB gene expression levels in the same condition, following double delta Ct method. Error bars represent the standard deviations of three independent replicates, each one measured three times

Supplementary Files

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