Concurrent methylation and demethylation of arsenic in fungal cells

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ABSTRACT: Microbial methylation and demethylation are central to arsenic's (As) biogeochemical cycling. Here, the transformations of monomethylarsonic acid (MMA(V)) (50 mg L^{-1}) for 15 days in cells of As-methylating fungi, *Fusarium oxysporum* CZ-8F1, *Penicillium janthinellum* SM-12F4, and *Trichoderma asperellum* SM-12F1, were evaluated, and trace concentrations of As(III) and As(V) were observed in fungal cell extracts. Trace amounts of DMA(V) were also detected in MMA(V) and *P. janthinellum* SM-12F4 incubations. In situ X-ray absorption near edge structure (XANES) indicated that after exposure to MMA(V) (500 mg L^{-1}) for 15 days, 28.6–48.6% of accumulated As in fungal cells was DMA(V), followed by 18.4–30.3% from As(V), 0–28.1% from As(III), and 4.8–28.9% from MMA(V). The concurrent methylation and demethylation of As occurs in fungal cells. The findings of this study will develop our understandings of microorganisms that drive As speciation transformation.

1 INTRODUCTION

Microbial methylation and volatilization of arsenic (As) drives As biogeochemical cycling ecosystems. Notably, organic matter, moisture, and pH all regulate As methylation and volatilization. Demethylation of methylarsenicals has been also widely studied in soils. However, there are very limited studies associated with As demethylation in microbial cells. Furthermore, some As-methylating microorganisms are capable of demethylation of methylarsenicals. *NsarsI* cloned from As-methylating *Nostoc* sp. PCC 7120 encodes a C-As lyase responsible for MMA(III) demethylation (Yan *et al.*, 2015). However, whether methylation and demethylation can be triggered concurrently in the same microbial cell remains unknown. Three fungal strains, *F. oxysporum* CZ-8F1, *P. janthinellum* SM-12F4, and *T. asperellum* SM-12F1, were shown to be capable of As(III) or As(V) methylation, with MMA(V) and DMA(V) generated as products (Su *et al.*, 2012). In this study, after exposure to MMA(V), As speciation transformation in *F. oxysporum* CZ-8F1, *P. janthinellum* SM-12F4, and *T. asperellum* SM-12F1 cells was evaluated using in situ X-ray absorption spectroscopy (XAS) and ion exchange chromatographic separation.

2 METHODS/EXPERIMENTAL

2.1 As speciation in fungal culture system using ion exchange chromatography separation

Briefly, after incubation with the fungal spore suspension (0.2 mL , 10^4 cfu mL^{-1}) in potato-glucose-peptone (PGP) medium (spiked with MMA(V) of

50 mg L^{-1}) for 1, 3, 5, 10, and 15 days, the culture medium was removed via centrifugation ($12,000 \text{ g}$, 15 min). A mixture of buffer solution ($0.1 \text{ M KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH 7.0, 10 mL) and ultrapure water (10 mL) was used to wash the compact fungal cells. Arsenic speciation in fungal cells was extracted using tetramethylammonium hydroxide (TMAH, 25%, 0.3 mL) and ultrapure water (4.7 mL) by grinding, centrifugation ($12,000 \text{ g}$, 15 min), and filtration (diameter, $0.20\text{-}\mu\text{m}$). Arsenic speciation analysis was conducted using high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS, SA-10, Titan Instruments, Beijing, China). PGP medium spiked with MMA(V) and without fungi was used to investigate the natural variance of MMA(V). Three replicates were run for each sampling time. No As was detected in the spore suspension used as the fungal inoculum.

2.2 As speciation in fungal cells using in-situ XAS

Fungal cells were harvested after cultivation for 15 days in PGP medium spiked with 500 mg L^{-1} MMA(V) ($\text{CH}_3\text{AsNaO}_3$, Strem Chemicals, Inc. Newburyport, USA). Sample preparation and analysis using X-ray absorption near edge structure (XANES) was performed as previously reported (Su *et al.*, 2012). For As distribution mapping using X-ray fluorescence (XRF), each point was scanned for 2 s using a silicon/lithium (Si/Li) detector with a spot size of $3.08 \times 2.78 \mu\text{m}^2$ and a step size of $5 \mu\text{m}$. For As speciation determination using XANES, each site was scanned for 6 s using a spot size of $3.08 \times 2.78 \mu\text{m}^2$ from 11,850 to 11,920 eV with a 0.5 eV step size. During XANES

analysis, As standard compounds were prepared with high-purity (>94.5%) chemicals.

3 RESULTS AND DISCUSSION

3.1 As speciation in fungal cells using ion exchange chromatographic separation

MMA(V), a small amount of inorganic As, and DMA(V) were observed in fungal cells after being exposed to MMA(V). MMA(V) contents in the fungal cells initially increased and then decreased with cultivation time. Furthermore, a small amount of As(III), As(V), or DMA(V) were also observed in fungal cells. For *T. asperellum* SM-12F1, the converse varying trends for As(III) and As(V) were observed with time. When cultivation time was prolonged to 15 days, the contents of As(III) and As(V) were 0.6 and $3.8 \mu\text{g g}^{-1}$, respectively. For *P. janthinellum* SM-12F4, DMA(V) of 0.4 and $28.7 \mu\text{g g}^{-1}$ at a cultivation time of 2 and 3 days was observed, respectively. As(III) and As(V) contents reached 1.5 and $0.9 \mu\text{g g}^{-1}$ when the cultivation time extended to 15 days, respectively. For *F. oxysporum* CZ-8F1, As(III) and As(V) of 0.3 and $1.2 \mu\text{g g}^{-1}$ were observed at a cultivation time of 15 days, respectively. This indicates that MMA(V) demethylation into As(III) and As(V) and methylation into DMA occurred in fungal cells after exposure to MMA(V) (data see Su *et al.*, 2017).

3.2 As speciation in fungal cells using in-situ XAS

Arsenic accumulation in fungal cells was easily observed using XRF, as shown in Figure 1, after exposure to 500 mg L^{-1} of MMA(V) for 15 days. The XANES spectra for each fungal strain and the standards are shown in Figure 1D. Evaluation of the XANES spectra beyond the absorption edge shows differences in the 11,862–11,875 eV regions of the three fungal strains. DMA(V) and inorganic As were simultaneously observed in fungal cells after MMA(V) exposure. For *T. asperellum* SM-12F1, 48.6% of the accumulated As was DMA(V), followed by 28.1% As(III), 18.4% As(V), and 4.8% MMA(V). For *P. janthinellum* SM-12F4, 41.1% of the accumulated As was DMA(V), followed by 30.3% As(V), and 28.9% MMA. No As(III) was observed. However, for *F. oxysporum* CZ-8F1, 29.0% of the accumulated As was As(V), followed by 28.6% DMA(V), 26.3% MMA(V), and 16.2% As(III). MMA(V) remethylation and demethylation could be simultaneously triggered in fungal cells (data see Su *et al.*, 2017).

The concurrent methylation and demethylation of As may exist in fungal hyphae. The concurrent methylation and demethylation of As is supported by our observations using the ion exchange chromatographic separation and *in situ* XANES. Comparatively, XANES analysis over-estimated the methylation of MMA(V). The differences between both methods might be attributed to: (I) As exposure condition, as higher As exposure might help to emerge the methylated arsenide in biological cells; (II) measurement

Figure 1. X-ray fluorescence (XRF) images and X-ray absorption near edge structure (XANES) spectra of three fungal strains after exposure to 500 mg L^{-1} of MMA(V) for 15 days. A, B, and C: XRF images for SM-12F1, SM-12F4, and CZ-8F1; D, XANES spectra of four references and three fungal strains.

scales, XANES analysis is used to determine As species in a particular area of a sample. While the typical method produced the average value of As in samples; (III) sample preparation, since samples for XANES analysis are preserved in their native state. However, the mixed cellular sample is acquired by cellular lysis and centrifugation in advance of HPLC-HG-AFS analysis. Notably, these differences do not interfere with our observation that the concurrent methylation and demethylation of As exists in fungal cells. The typical method coupled with *in situ* XANES is recommended to detect As species in samples.

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REFERENCES

- Su, S.M., Zeng, X.B., Li, L.F., Duan, R., Bai, L.Y., Li, A.G., Wang, J. & Jiang, S. 2012. Arsenate reduction and methylation in the cells of *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1 investigated with X-ray absorption near edge structure. *J. Hazard. Mater.* 243: 364–367.
- Su, S.M., Zeng, X.B., Bai, L.Y., Wang, Y.N., Zhang, L.L., Li, M.S. & Wu, C.X. 2017. Concurrent methylation and demethylation of arsenic by fungi and their differential expression in the proteoplasm proteome. *Environ. Pollut.* 225: 620–657.
- Yan, Y., Ye, J., Xue, X.M. & Zhu, Y.G. 2015. Arsenic demethylation by a C-As lyase in cyanobacterium *Nostoc* sp. PCC 7120. *Environ. Sci. Technol.* 49: 14350–14358.