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Arsenic methylation by an arsenite S-adenosylmethionine methyltransferase from *Spirulina platensis*

Yuqing Guo^{1,2}, Ximei Xue², Yu Yan², Yongguan Zhu^{2,3}, Guidi Yang^{1,*}, Jun Ye^{2,*}

1. Fujian Provincial Key Laboratory of Agroecological Processing and Safety Monitoring, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China. E-mail: yqguo@iue.ac.cn

2. Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

3. State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

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ABSTRACT

Arsenic-contaminated water is a serious hazard for human health. Plankton plays a critical role in the fate and toxicity of arsenic in water by accumulation and biotransformation. *Spirulina platensis* (*S. platensis*), a typical plankton, is often used as a supplement or feed for pharmacy and aquaculture, and may introduce arsenic into the food chain, resulting in a risk to human health. However, there are few studies about how *S. platensis* biotransforms arsenic. In this study, we investigated arsenic biotransformation by *S. platensis*. When exposed to arsenite (As(III)), *S. platensis* accumulated arsenic up to 4.1 mg/kg dry weight. After exposure to As(III), arsenate (As(V)) was the predominant species making up 64% to 86% of the total arsenic. Monomethylarsenate (MMA(V)) and dimethylarsenate (DMA(V)) were also detected. An arsenite S-adenosylmethionine methyltransferase from *S. platensis* (SpArsM) was identified and characterized. SpArsM showed low identity with other reported ArsM enzymes. The *Escherichia coli* AW3110 bearing *SparsM* gene resulted in As(III) methylation and conferring resistance to As(III). The *in vitro* assay showed that SpArsM exhibited As(III) methylation activity. DMA(V) and a small amount of MMA(V) were detected in the reaction system within 0.5 hr. A truncated SpArsM derivative lacking the last 34 residues still had the ability to methylate As(III). The three single mutants of SpArsM (C59S, C186S, and C238S) abolished the capability of As(III) methylation, suggesting the three cysteine residues are involved in catalysis. We propose that SpArsM is responsible for As methylation and detoxification of As(III) and may contribute to As biogeochemistry.

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Introduction

Arsenic (As) is one of the most common contaminants in the environment. The average As concentration in marine waters is around 1.5 µg/L (Cullen and Reimer, 1989). While concentrations of As vary in freshwaters, depending on the geological features and the result of As mobilization through a combination of

natural processes as well as through a range of anthropogenic activities (Smedley and Kinniburgh, 2002), As-contaminated water is causing worldwide drinking water safety and food supply problems as around 150 million people are exposed to As-contaminated drinking water, particularly in South or Southeast Asia (Brammer and Ravenscroft, 2009; Christen, 2001; Meliker et al., 2007; Pan et al., 2014). In addition to drinking

* Corresponding authors. E-mail addresses: guidiyang@fafu.edu.cn (Guidi Yang), jye@iue.ac.cn (Jun Ye).

water, As biomagnification by alga, fishes, and other aquatic organisms also introduces As into the food chain and can be a potential source of threat to human health (Maher et al., 2011; Rahman et al., 2012).

Arsenic biotransformation by organisms plays an important part in its fate in the environment and toxicity to cells (Ye et al., 2012). Arsenic is primarily present in the form of inorganic As in natural environments, and inorganic As can be biotransformed into organic As, such as methylated arsenicals, arsenosugars, and arsenolipids. The mechanisms of As biotransformation including oxidation, reduction, and methylation have been widely studied. Arsenite (As(III)) oxidation is believed to be a detoxification pathway since arsenate (As(V)) is less toxic than As(III) (Armendariz et al., 2015; Paez-Espino et al., 2009). Although the possible intermediate products methylarsenite (MMA(III)) and dimethylarsenite (DMA(III)) are more toxic, As methylation is acknowledged as a pathway of detoxification in microbes (Styblo et al., 2002; Wang et al., 2014b). One classical As biomethylation scheme can be summarized as alternating reduction and methylation, producing less toxic methylated arsenicals such as methylarsenate (MMA(V)), dimethylarsenate (DMA(V)), and trimethylarsine oxide (TMAO(V)) (Bentley and Chasteen, 2002; Challenger, 1945). Recent studies proposed alternative pathways in which MMA(III) and DMA(III) remain trivalent throughout the whole catalytic cycle (Hayakawa et al., 2005; Marapakala et al., 2012).

S. platensis is a cyanobacterium that forms massive populations in freshwater, brackish condition, and in some marine environments (Vonshak, 1997). It has been used as food by the Aztecs in the 16th century (Del Castillo, 1956), and was rediscovered in the mid-1960s (Vonshak, 1997). *S. platensis* is rich in protein, vitamins, and essential fatty acids (Babadzhanov et al., 2004; Ciferri, 1983), so it is an attractive food source for less-developed countries where people still suffer from famine problems. Nowadays, it is often used as a commercial medicine (as pills, tablets, or capsules) recommended as a supplement of vitamins and proteins (Campanella et al., 1998) or applied as a feed in aquaculture (Vonshak, 1997). In a word, it has a basal position in the food web, and plays an important role in the ecosystem. However, the total As concentration of *Spirulina* supplement from Mexico and Chad was detected to exceed the maximum limit of the National Health Surveillance Agency of Brazil (Richmond and Hu, 2013). The heavy metal excess of edible *Spirulina* has attracted increasing attention, and the heavy metal pollution might come from breeding, drying, and processing. At the same time, *S. platensis* can rapidly accumulate heavy metals, for example the maximum sorption capacity of As(V) reached up to 525 mg/g (Doshi et al., 2009). This indicated that *S. platensis* in the natural environment might be an effective bioremediation material for removing heavy metals from water (Disyawongs, 2002; Doshi et al., 2009; Lodi et al., 2008; Solisio et al., 2006). Therefore, studies about accumulation and biotransformation of As by *S. platensis* are needed, since only total As was measured in previous studies.

Thus, we investigated the molecular mechanism of As biotransformation by *S. platensis* by treating it with As(III). Furthermore, a gene encoding the As(III) methyltransferase (SpArsM) was identified, and the SpArsM protein was purified and characterized.

1. Materials and methods

1.1. Organisms and cultivations

S. platensis FACHB-439 was obtained from the Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China and incubated in modified Zarrouk's medium (Seshadri and Thomas, 1979). The growth condition for *S. platensis* was under a 12-hr light–dark cycle with a light intensity of approximately 50 $\mu\text{mol}/(\text{m}^2\cdot\text{sec})$, 26°C (day)/25°C (night). During incubation, the cultures were shaken by hand twice a day. *E. coli* cells were grown in Luria-Bertani (LB) medium at 37°C with corresponding antibiotics. Strain DH5 α (TransGen) was used for plasmid construction. Strain AW3110 (ΔarsRBC) (Carlin et al., 1995) was used for functional verification of the *arsM* gene. Strain Transetta (DE3) (TransGen) was used for protein expression and purification.

1.2. Arsenic species in *S. platensis*

S. platensis FACHB-439 in the exponential phase were treated with indicated concentrations of As(III) for 15 days. CK treatments (1 $\mu\text{mol}/\text{L}$ As(III) without bacteria) were placed under the same conditions. Trimethylarsenite (TMA(III)) released from cyanobacteria was collected as reported previously (Yin et al., 2011). As species was determined to investigate biotransformation of As, and the extraction method of cellular As was performed as described before (Yan et al., 2015).

1.3. Cloning of SparsM from *S. platensis*

The genomic DNA of *S. platensis* FACHB-439 was extracted by using a DNA extraction kit (Bioteke) according to the manufacturer's instructions. As the genome of *S. platensis* FACHB-439 has not been sequenced, we chose the putative *arsM* in *Arthrosira platensis* (GenBank accession number WP_006619316.1) to assist in the design of primers. An 1185-bp fragment was successfully amplified by PCR with primers 5'-CATATGACTCCAGCTAAATC GGCAAATACTGC-3' (*NdeI* site underlined), and 5'-CTCGAGACA ACAACTTGATGGAGAAC-3' (*XhoI* site underlined). The PCR product was cloned into the pMD19-T vector (Takara) to create plasmid pMD19-T-SparsM. After extracting with a plasmid extraction mini kit (Tiangen), the plasmid was digested with *NdeI* and *XhoI* and inserted into pET22b vector to create the plasmid pET22b-SparsM. The pET22b-SparsM was subsequently transformed into the *E. coli* Transetta (DE3) or AW3110 (ΔarsRBC). The His-tagged SpArsM was composed of 401 amino acids with a mass of 45 kDa. By carrying out multiple-sequence alignment of arsenite S-adenosylmethionine methyltransferase (ArsM) orthologs, we found that the N-termini and C-termini are not conserved, and an N- and C-terminally truncated derivative of ArsM from *Tetrahymena pyriformis* did not lose the ability to methylate As(III) (Ye et al., 2014). To verify whether the last 34 residues of SpArsM affected As(III) methylation, the truncated derivative of SpArsM (*arsM34*) was also constructed by using primers 5'-CATATGACTCCAGCTAAATCGGCAAATACTG-3' (*NdeI* site underlined) and 5'-CTCGAGACAATCAAATCTTTAGCATC CTTTAG-3' (*XhoI* site underlined), resulting in a protein of 367 amino acids with a mass of 41 kDa.

1.4. Resistance assays in *E. coli*

Arsenic resistance assays were performed as previously reported (Qin et al., 2006). Single colonies of *E. coli* AW3110 (Δ arsRBC) bearing pET22b and pET22b-SpArsM (full-length) were inoculated into 5 mL LB medium containing 100 μ g/mL Ampicillin and 30 μ g/mL Chloramphenicol. After incubating at 37°C overnight with shaking at 180 r/min, the late exponential phase cells were diluted 100-fold into 40 mL fresh LB medium containing the antibiotics, 0.3 mmol/L isopropyl β -D-1-thiogalactopyranoside (IPTG) and the indicated concentrations of As(III). Growth was monitored as optical density at 600 nm by using an ultraviolet-visible spectrophotometer (Mapada).

1.5. Arsenic methylation assays in *E. coli*

As(III) methylation was measured in *E. coli* AW3110 (Δ arsRBC) bearing either plasmid pET22b-SpArsM or empty vector pET22b. Cultures were grown overnight in LB medium containing 100 μ g/mL Ampicillin and 30 μ g/mL Chloramphenicol, then diluted 100-folds into 5 mL fresh LB medium containing the corresponding antibiotics. Final concentrations of 0.3 mmol/L IPTG and 10 μ mol/L As(III) were added when cells reached exponential phase. Culture samples were centrifuged at 12,000 r/min for 1 min after 19 hr incubation. The supernatants were filtrated through 0.22 μ m filters and then stored at –20°C until As species were analyzed. Volatile As produced by SpArsM was collected as described (Huang et al., 2012).

1.6. Overexpression and purification of SpArsM

Cells of Transetta (DE3) bearing pET22b-SpArsM or pET22b-SpArsM34 were incubated at 37°C in LB media, 0.3 mmol/L IPTG was supplemented to induce expression of His-tagged SpArsM or SpArsM34 when OD₆₀₀ reached 0.4–0.6. After continuing to incubate at 18°C overnight, cells were harvested by centrifugation (6000 r/min) at 4°C for 15 min and then suspended in buffer A (20 mmol/L HEPES, 0.5 mol/L NaCl, 3 mmol/L tris(2-carboxyethyl)phosphine (TCEP), pH 7.4). The cells were lysed by a single pass through a French-press at 10 MPa and the lysate was centrifugated at 19,000 r/min for 45 min to remove cell debris and unbroken cells. The supernatant was filtrated through a 0.22 μ m filter and loaded onto a Ni-NTA agarose column (Qiagen) pre-equilibrated with buffer A. The column was washed by 100 mL buffer B (20 mmol/L HEPES, 0.5 mol/L NaCl, 20 mmol/L imidazole and 3 mmol/L TCEP, pH 7.0) and finally eluted with 15 mL buffer C (20 mmol/L HEPES, 0.5 mol/L NaCl, 500 mmol/L imidazole and 3 mmol/L TCEP, pH 7.0). Fractions containing SpArsM or SpArsM34 were concentrated using a 10-kDa-cutoff Amicon Ultrafilter (Millipore). Protein concentrations were determined by absorbance at 280 nm. SpArsM and SpArsM34 proteins were checked by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

1.7. As(III) methylation assay using purified SpArsM protein

As(III) methylation in vitro assays with purified SpArsM or SpArsM34 were performed in buffer D (50 mmol/L MOPS and

125 mmol/L KCl, pH 7.5) containing 8 mmol/L GSH, 1 mmol/L SAM, 5 μ mol/L ArsM and 10 μ mol/L As(III) at 37°C for 12 hr. The reactions were terminated by boiling for 5 min. Some of the reaction solutions were oxidized by 30% (V/V) H₂O₂. The changes of As species during the reactions were monitored at indicated time (0, 0.5, 1, 3, 6, 12 hr).

1.8. Preparation of SpArsM mutants

Mutations of SpArsM were generated by site-directed mutagenesis of pET22b-SpArsM using primers listed in Appendix A Table S1. Three single mutants of SpArsM (C59S, C186S, or C238S) were generated by changing codons for cysteine to serine. Each mutation was confirmed by sequencing. The plasmid vectors carrying different mutants were transformed into *E. coli* AW3110.

1.9. Arsenic speciation analysis

Arsenic speciation was analyzed by high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP–MS) (Agilent 1200 series and Agilent 7500 series, Agilent Technologies, USA) as previously described (Zhu et al., 2008). A Hamilton PRP-X100 10- μ m anion-exchange column (250 $\times$ 4.1 mm²) was used for chromatography. The mobile phase consisted of 10 mmol/L diammonium hydrogen phosphate ((NH₄)₂HPO₄) and 10 mmol/L ammonium nitrate (NH₄NO₃) and adjusted to pH 6.2 with nitric acid, at a flow rate of 1.0 mL/min. The analytical method was validated through analyses of GBW10025-a spiral seaweed (certified [total As] = 0.22 $\pm$ 0.03 mg/kg, certified [total Cr] = 1.50 $\pm$ 0.13 mg/kg, found [total As] = 0.26 $\pm$ 0.03 mg/kg, and [total Cr] = 1.63 $\pm$ 0.16 mg/kg).

2. Results

2.1. Arsenic methylation by *S. platensis*

In order to investigate whether *S. platensis* is capable of As methylation under laboratory conditions, *S. platensis* was cultured in Zarrouk's medium under exposure to different concentrations of As(III) for 15 days and the As species in cells were determined. As(V) was the dominant intracellular species accounting for 64% to 86% of the total arsenic (Fig. 1). About 2% to 15% of the total arsenic were in the form of methylated arsenic species including DMA(V) and MMA(V) after exposure to the indicated concentrations of As(III). This showed that *S. platensis* was able to accumulate and methylate As. No volatile As was detected.

2.2. Cloning of SpArsM

PCR cloning using total DNA extracts from *S. platensis* yielded one *arsM* gene, SpArsM. SpArsM was 97% identical to the putative product of *arsM* from *Arthrospira platensis*. Multiple-sequence alignment showed that SpArsM shared 39%, 33%, and 35% identity with the annotated arsenite methyltransferase proteins in *Methanosarcina mazei* Go1 (GenBank accession no. WP_011032612.1), *Rhodospseudomonas palustris* CGA009 (GenBank accession no. WP_011159102), and *Aspergillus fumigatus* (GenBank accession no. XM_748062), respectively. It also exhibited lower

Fig. 1 – Water-soluble arsenic species in *S. platensis* after exposure to indicated concentrations of As(III) for 15 days, DW: dry weight.

identity to eukaryotic *Cyanidioschyzon* sp. 5508 ArsM (GenBank accession no. ACN39190), the AS3MT arsenite methyltransferases from human (Genbank accession no. AAI19639.2), and *Danio rerio* (GenBank accession no. NP_001034928.1) (32%, 27%, and 26% identity, respectively). In addition, SpArsM only showed 26% and 27% identity with the two cyanobacterial *Synechocystis* sp. PCC 6803 and *Nostoc* sp. PCC 7120 ArsMs, respectively. The phylogenetic analysis (Appendix A Fig. S2) showed that the three cyanobacterial ArsMs belong to two different groups, the reason for the distant relationship between the three is not clear.

2.3. SpArsM conferred resistance to As(III) in *E. coli*

To examine whether SpArsM enhances As resistance, the *SparsM* gene was expressed in As-sensitive *E. coli* AW3110 (Δ arsRBC) which had no orthologous *arsM* gene. At 70 μmol/L or 90 μmol/L As(III), cells bearing the *SparsM* gene grew much better than those bearing pET22b (Fig. 2), demonstrating that SpArsM conferred resistance to As(III). In addition, AW3110 bearing *SparsM* grew slightly better than the control in the absence of As(III).

2.4. As(III) methylation by *E. coli* AW3110 expressing *SparsM*

To test As(III) methylation activity of SpArsM *in vivo*, *E. coli* strain AW3110 bearing *SparsM* or pET22b was incubated with 10 μmol/L As(III) for 19 hr, and As species were analyzed (Fig. 3). Methylated arsenicals were not detected in the medium containing *E. coli* AW3110 bearing pET22b. Meanwhile, we found that the increase of DMA(V) concentration was coincident with the decrease of As(III) in the medium containing *E. coli* AW3110 expressing *SparsM*. DMA(V) was the predominate product, accounting for 84% of total As. In addition, small amounts of MMA(V) were also detected in the medium, while no volatilized As was detected no matter what concentration of As(III) (1, 10, 70, or 90 μmol/L) was added into the media (data was not shown). This result showed SpArsM had the ability of

Fig. 2 – Expression of *SparsM* conferred arsenite resistance in *E. coli* AW3110. Cellular growth was monitored as absorbance at 600 nm at indicated time points. The data represent the average of 3 replicate cultures.

As(III) methylation, but the end product was DMA(V), not TMA(III).

2.5. *In vitro* As(III) methylation using purified SpArsM and SpArsM34

To further elucidate the function of SpArsM and SpArsM34 in As(III) methylation, both *SparsM* and *SparsM34* were expressed in *E. coli* Transetta and purified in a single step by Ni(II)-nitrilotriacetic acid (NTA) chromatography. Purified SpArsM protein showed As(III) methylation activity at pH values between 2.5 and 9.5, with the highest As(III) methylation activity being observed at pH 7.5 (Appendix A Fig. S3B). Furthermore, SpArsM could convert almost all As(III) to DMA(V) at 16°C and 37°C, while the rate of As(III) methylation was reduced at 60°C, resulting in more MMA(V) than DMA(V) being produced (Appendix A Fig. S3C). The time-dependent methylation products generated by SpArsM under optimal reaction condition (37°C and pH 7.5) were also analyzed (Fig. 4A). As(III) methylation to DMA(V) was observed within

Fig. 3 – The As species in the medium incubating *E. coli* AW3110 expressing *SparsM* for 19 hr at 37°C.

Fig. 4 – As(III) methylation by purified SpArsM and SpArsM34. (a) Time course of As(III) methylation by purified SpArsM. (b) As species of As(III) methylation by purified SpArsM and SpArsM34 in MOPS buffer (pH 7.5) at 37°C for 12 hr.

0.5 hr. In order to verify whether the C-terminal residues of SpArsM would have an impact on As(III) methylation, *in vitro* catalysis of the truncated SpArsM derivative (SpArsM34) was also performed under the same condition. Results showed SpArsM34 was also able to methylate As(III) to DMA(V) (Fig. 4B), and the catalytic activity of SpArsM34 was higher than SpArsM.

2.6. Conserved cysteine residues Cys59, Cys186, and Cys238 of SpArsM

Conserved cysteine residues in As(III) methyltransferases are critical in catalyzing As methylation (Marapakala et al., 2012; Qin et al., 2006; Song et al., 2011). The amino acid sequence of SpArsM contains 16 cysteine residues, and Cys59, Cys186, and Cys238 are the conserved cysteines as detected by multiple alignment. Herein, the three cysteine residues were individually altered to serine by site-directed mutagenesis to create C59S, C186S, and C238S derivatives. In order to test whether the three cysteines are involved in As(III) methylation, the *in vivo* As methylation ability of these three mutants expressed in *E. coli* AW3110 were studied. All of the three SpArsM mutants completely abolished the As(III) methylation capability, and no methylated arsenic species were detected in the

medium (Fig. 5). These results indicated that each of the three conserved cysteine residues was required for As(III) methylation.

3. Discussion

Arsenic is ubiquitous in aquatic environments where various kinds of cyanobacteria are distributed (Qin et al., 2006, 2009; Zhang et al., 2013), so cyanobacteria have evolved several ways to detoxify As in order to survive in the environment (Aurilio et al., 1994; Knauer et al., 1999). In this study, our results showed that *S. platensis* could grow at high concentrations of the toxic metalloid and had the capability of As(III) accumulation and biotransformation. In general, As(III) is detoxified directly by efflux out of cells, oxidation to As(V), or methylation to methylated As species (Bentley and Chasteen, 2002). Here, we found that these parallel pathways of As(III) detoxification exist in *S. platensis*. When *S. platensis* was exposed to As(III), the main As species was As(V) in *S. platensis* which indicated As(III) oxidation may be the primary As(III) detoxification pathway. This is similar to the response of other cyanobacteria, such as *Synechocystis* sp. PCC6803 (Yin et al., 2011), which oxidizes As(III) to As(V) for detoxification. Moreover, *S. platensis* showed the ability of As(III) methylation after a long-time As(III) exposure. As methylation is one of the crucial processes governing the fate of As in the environment. It is necessary to understand the mechanism of the different As methyltransferases. The As methylation pathway has been studied for a long time. However, the ArsMs responsible for As(III) methylation were identified and characterized only in recent years (Lin et al., 2002). We found an ArsM orthologue from *S. platensis*, and verified that it is responsible for As(III) biotransformation. Both *E. coli* bearing the *SparsM* gene and purified SpArsM protein conferred the ability to methylate As(III). These results clearly suggest that ArsM-mediated As

Fig. 5 – As(III) methylation *in vivo* assay of *E. coli* AW3110 expressing wild-type and mutants of SpArsM in the presence of 10 μmol/L As(III) at 37°C for 24 hr.

methylation in *S. platensis* is a possible detoxification mechanism.

Although As biomethylation has been widely studied in bacteria, archaea, and fungi and the end product always was the volatilizable TMA(III) (Challenger, 1945; Hayakawa et al., 2005; Marapakala et al., 2012), we found *S. platensis* cannot generate TMA(III), and As(III) methylation of SpArsM ended with DMA(V) as the final product in both in vivo and vitro assays. This is similar to results in zebra fish (Hamdi et al., 2012). As(III) undergoes sequential methylations producing mono-, di-, and trimethylated species by ArsM, and the last methylation reaction to generate TMA(III) from DMA(V) is proposed to be the rate-limiting step (Marapakala et al., 2012). Herein, we suggest that the last reaction of As(III) methylation was not catalyzed by SpArsM, and DMA(V) might be used to produce arsenosugars or arsenolipids in *S. platensis*. According to the result of multiple-sequence alignments, SpArsM exhibited low similarity to other ArsMs from cyanobacteria with the sequence identity to *Synechocystis* sp. PCC 6803 and *Nostoc* sp. PCC 7120 ArsM being only 26% and 27%, respectively. The huge difference between the sequences may explain the difference in methylation products. In addition, As(III) was seen to be transformed to DMA(V) within 30 min, and the catalytic rate of SpArsM was much faster than that of other ArsMs (Qin et al., 2009; Wang et al., 2014a; Zhang et al., 2015). Meanwhile, the intermediate MMA(III) and MMA(V) were absent in the in vitro enzyme activity assays when the reaction was performed at optimal conditions, and only MMA(V) was observed when the enzyme was impaired, for example under higher or lower temperature. This result may possibly be due to MMA strongly binding to SpArsM or the methylation process from MMA to DMA was so quick that little MMA was released into the reaction medium.

N- or C-terminal residues of enzymes related to arsenic resistance are usually not required for their functions, while cysteine residues are often conserved and play an important role in the structure and function of the enzymes (Dheeman et al., 2014; Fomenko et al., 2008; Marapakala et al., 2012). The multiple-sequence alignment of ArsM illustrates that three cysteine residues are highly conserved and were shown to be involved in As(III) binding and catalysis of As methylation by site-directed mutagenesis (Ajees et al., 2012; Dheeman et al., 2014; Marapakala et al., 2012, 2015). Herein, we constructed a C-terminal truncated SpArsM and three cysteine mutants (C59S, C186S, C238S) to investigate whether deleting or mutating these residues had an effect on the function of SpArsM. Our results demonstrate that SpArsM34 still showed the ability of As methylation, and this result was not surprising because other truncated ArsMs from *Tetrahymena pyriformis* (30TpyarsM8) (Ye et al., 2014) and *Cyanidioschyzon* sp. 5508 (Ajees et al., 2012) did not lose their ability to methylate As(III). In our study, the catalytic rate of SpArsM34 increased compared to the full-length SpArsM. This result indicated that the truncation of amino acids may facilitate the stability of protein structure which results in an increase of enzyme activity in vitro (Beckstead et al., 2005). Moreover, neither of the single cysteine mutants of SpArsM retained the ability to methylate As(III), which indicated that Cys59, Cys186, and Cys238 of SpArsM are essential in maintaining the function. The three cysteines are conserved in all of the ArsMs ranged

from bacteria to humans, and more work should be done to elucidate the evolution of ArsM.

In conclusion, our results clearly showed that *S. platensis* was able to accumulate and biotransform As(III), and the molecular mechanism of As methylation by SpArsM was also elucidated. *S. platensis* is not only a crucial part of the food chain in aquatic environments but also can be a direct source of As exposure for humans by being used as a health supplement. The potential risk of *S. platensis* regarding arsenic accumulation may have been overlooked. Our results may help understand the role of *S. platensis* in As biogeochemistry in the environment, and could be further used to evaluate the risk of *S. platensis* in the food chain and to explore the possibility to be applied as a bioremediation material to remove As from As-contaminated water.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jes.2016.06.013>.

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