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Arsenic biotransformation by a cyanobacterium *Nostoc* sp. PCC 7120☆

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ABSTRACT

Nostoc sp. PCC 7120 (*Nostoc*), a typical filamentous cyanobacterium ubiquitous in aquatic system, is recognized as a model organism to study prokaryotic cell differentiation and nitrogen fixation. In this study, *Nostoc* cells incubated with arsenite (As(III)) for two weeks were extracted with dichloromethane/methanol (DCM/MeOH) and the extract was partitioned between water and DCM. Arsenic species in aqueous and DCM layers were determined using high performance liquid chromatography - inductively coupled plasma mass spectrometer/electrospray tandem mass spectrometry (HPLC-ICPMS/ESIMSMS). In addition to inorganic arsenic (iAs), the aqueous layer also contained monomethylarsonate (MAs(V)), dimethylarsinate (DMAs(V)), and the two arsenosugars, namely a glycerol arsenosugar (Oxo-Gly) and a phosphate arsenosugar (Oxo-PO4). Two major arsenosugar phospholipids (AsSugPL982 and AsSugPL984) were detected in DCM fraction. Arsenic in the growth medium was also investigated by HPLC/ICPMS and shown to be present mainly as the inorganic forms As(III) and As(V) accounting for 29%–38% and 29%–57% of the total arsenic respectively. The total arsenic of methylated arsenic, arsenosugars, and arsenosugar phospholipids in *Nostoc* cells with increasing As(III) exposure were not markedly different, indicating that the transformation to organoarsenic in *Nostoc* was not dependent on As(III) concentration in the medium. Our results provide new insights into the role of cyanobacteria in the biogeochemical cycling of arsenic.

1. Introduction

Arsenic is a ubiquitous and carcinogenic toxic element, and has both acute and chronic toxicity effects on humans. The bioavailability of arsenic and its resultant toxicity are influenced to a great extent by its species (Smedley and Kinniburgh, 2002; Sharma and Sohn, 2009). Inorganic arsenic, the major form of arsenic in water and soils, is transformed into organic arsenic species or in reverse in natural biological processes, and microorganisms play a critical role in arsenic biogeochemical cycle (Zhu et al., 2014; Zhang et al., 2017).

To survive in arsenic-enriched environments, organisms have evolved various mechanisms to utilize or detoxify arsenic (Stolz et al., 2006). Following the absorption of As(V) into cells via phosphate transport systems, a two-step pathway is invoked by organisms to decrease arsenic levels in cells. As(V) is first reduced to As(III), and then As(III) is either pumped out via membrane proteins or stored in vacuoles (Tamaki and Frankenberger, 1992). Methylated arsenic species are also widely found in organisms, especially in many photosynthetic organisms (Ye et al., 2012). Although trivalent methylated arsenic, methylarsonous acid (MAs(III)) and dimethylarsinnous acid (DMAs(III)), are much more toxic than As(III), As(III) methylation is considered as a process of detoxification eventually producing MAs(V), DMAs(V), and trimethylarsine oxide (TMAO) (Qin et al., 2006, 2009; Yin et al., 2011). Man-made organoarsenic (e.g. herbicide, roxarsone, nitarsone, and *p*-arsanilic acid) are widely used in the agriculture, and could be biodegraded to inorganic arsenic by microbes, worsening food and water pollution (Yoshinaga and Rosen, 2014; Yoshinaga and Cai, 2011; Chen and Rosen, 2016).

Other, more complex, organoarsenic compounds such as arsenobetaine, arsenosugars and arsenolipids are also found in

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marine (Francesconi, 2005), freshwater (Miyashita et al., 2009, 2012), and terrestrial organisms (Geiszinger et al., 1998). So far, more than 20 arsenosugars have been characterized (Nischwitz and Pergantis, 2007), and arsenolipids are also now often reported in marine samples (Glabonjat et al., 2014; Rumpler et al., 2008; Taleshi et al., 2010). However, the biosynthetic processes leading to the production of arsenosugars or arsenolipids are still not well understood.

Cyanobacteria are involved in arsenic biogeochemical cycle, and have been reported to have the ability to methylate inorganic arsenic (Ye et al., 2012; Guo et al., 2016), produce arsenosugars and arsenosugar phospholipids (Xue et al., 2014a). The previous studies showed that *Nostoc* methylated As(III) to DMAs(V) and TMA(O) (Yin et al., 2011), demethylated MAs(V) and MAs(III) into As(III) (Yan et al., 2015), and produced Oxo-Gly (Miyashita et al., 2012). Compared to the extraction with water or nitric acid, the extraction with dichloromethane/methanol (DCM/MeOH) will divide arsenic in cells into water-soluble (such as inorganic arsenic, methylated arsenic, arsenosugars, etc) and lipid-soluble arsenic (such as arsenosugar phospholipids, arsenic-containing fatty acids, arsenic-containing hydrocarbons, etc) (Glabonjat et al., 2014). In this study, HPLC-ICPMS/ESIMS was used to analyze water-soluble and lipid-soluble arsenic species of *Nostoc* in order to understand arsenic biotransformation by *Nostoc* from multiple perspectives. The Oxo-PO_4 and two arsenosugar phospholipids were found in *Nostoc* treated with As(III). For the first time, arsenic demethylation and arsenolipid biosynthesis were found to co-occur in one organism. It has great implications for future studies aiming to fully understand arsenic transformation and fate in the environment.

2. Materials and methods

2.1. Cyanobacteria culture and harvesting

Axenic cultures of *Nostoc* were grown in 150 mL Erlenmeyer flasks containing 50 mL BG-11 medium without $NaNO_3$ (Rippka et al., 1979) at 28 °C with shaking at 120 rpm under continuous light (40 μmol photons m^{-2} s^{-1}). *Nostoc* cells at stationary phase (cell number remains constant during this phase) were treated with 0.1, 1, 10, 100 μM As(III) for two weeks; each treatment was performed in triplicate. *Nostoc* was harvested by centrifuging at 4700 g for 15 min at 4 °C. The sample was separated into two parts: growth medium and cells. The growth medium was filtered using syringe filters (0.2 μm Nylon membrane) (VWR International, West Chester, PA, USA), and stored at −80 °C until arsenic species and total arsenic analysis. The cells were transferred to 15 mL polypropylene tubes with screw-caps after being washed three times with cold MES buffer (Yin et al., 2011), then freeze-dried.

2.2. Fractionation of arsenic in Nostoc

The fractionation of arsenic in *Nostoc* was carried out as described in Fig. 1. About 30 mg of freeze-dried cells were weighed (to a precision of 0.1 mg) directly into a centrifuge tube (15 mL, polypropylene), and 5 mL of a mixture of DCM/MeOH (2 + 1, v/v) was added. The mixture was extracted on a rotary wheel overnight, and centrifuged at 4754 g and 4 °C for 15 min.

0.5 mL of 1% aqueous NH_4HCO_3 solution was added to the supernatant (~4.5 mL), and the mixture was gently shaken. After standing for 30 min, the solution was separated into an aqueous layer (upper layer, MeOH and H_2O) and DCM-MeOH layer (lower layer). The aqueous layer was transferred to 2 mL Eppendorf tubes, and stored at −80 °C for later total arsenic analysis and water-soluble arsenic species determination.

A portion of the DCM layer (2 mL) was applied to a 150 × 5 mm glass Pasteur pipet filled with silica gel 60 (to a height of 4 cm) that was pre-conditioned with 5 mL of 1% formic acid in acetone/MeOH (1 + 1, v/v). The column was washed with pure MeOH (5 mL) firstly, then the arsenolipids were eluted with 5 mL of pure MeOH containing 1% $NH_4 \cdot H_2O$. The arsenolipid fraction was evaporated to dryness, and stored at 4 °C until analysis. 300 μL of ethanol (EtOH) were added to the arsenolipid fraction, and ultrasonicated for 30 min 200 μL of the arsenolipid fractionwas used for analysis after centrifuging for 15 min and filtering with 0.2 μm Nylon filters (ProFills, Markus Bruckner Analysentechnik, Linz, Austria).

The pellet after DCM/MeOH extraction, dried using nitrogen stripping method, was added with 500 μL of H_2O, sonicated for 15 min, and the mixture subjected to repetitive freeze-thawing (6 cycles) before being centrifuged for 15 min. Arsenic species and total arsenic concentration in the supernatant were analyzed. The residue after water extraction was dried for determination of total arsenic concentration.

2.3. Determination of total arsenic concentration

The lyophilized *Nostoc* (about 15 mg), part of all sample extracts, the residue after water extraction, and CRM 7405-a (certified reference material) were analyzed for total arsenic concentration as follows. The samples were weighed into polypropylene digest tubes (50 mL), and 2 mL of a solution of internal standards (100 μg L^{-1} Ge, In, Te in 1% HNO_3) and 2 mL conc. HNO_3 were added. The samples were transferred to a microwave-accelerated reaction system (Mars CEM, CEM Corporation, Matthews, NC, USA), and digested according to the following temperature program: 0–10 min, 80 °C; 10–30 min, 120 °C; 30–60 min, 160 °C. The clear digest solutions were transferred to polypropylene tubes (15 mL), and diluted with Mill-Q water to 10 mL after being cooled to room temperature.

Determination of total arsenic concentration in the digested solutions were carried out using an Agilent 7500cx ICP-MS in collision cell mode (He, 5 mL min^{-1}) to avoid interferences from $^{40}Ar^{35}Cl$ on ^{75}As. The digest and analytical methods were validated against the certified reference material CRM 7405-a (Hijiki), with a certified value for arsenic of 35.8 ± 0.9 mg As kg^{-1}; we obtained 36.2 ± 0.8 mg As kg^{-1} (n = 3) in this work.

2.4. HPLC-ICPMS/ESIMSMS analysis of water-soluble arsenic species

Anion-exchange HPLC (Agilent 1100 HPLC system) was carried out with a PRP-X100 column (4.6 × 150 mm; 5 μm particle size;

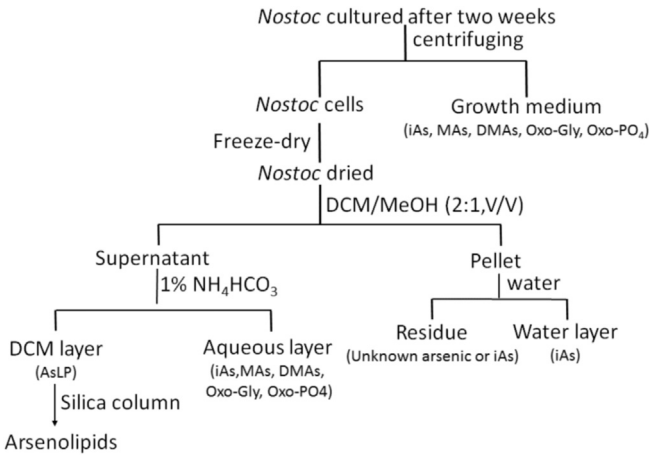

Fig. 1. The diagram of extracting arsenic compounds from *Nostoc*.