Multiple species of arsenic biotransformation occur in *Nostoc* sp. PCC 7120

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ABSTRACT: *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.

1 INTRODUCTION

Arsenic (As) 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.¹ 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).

Cyanobacteria are involved in arsenic biogeochemical cycle, and have been reported to have the ability to methylate inorganic arsenic, producing arsenosugars and arsenosugar phospholipids. 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). In this study, HPLC-ICPMS/ESIMS was used to analyze arsenic biotransformation in *Nostoc* in order to understand arsenic biotransformation by *Nostoc* from multiple perspectives.

2 METHODS/EXPERIMENTAL

2.1 Fractionation of arsenic in *Nostoc*

About 30 mg of freeze-dried cells were weighed (to a precision of 0.1 mg) directly into a centrifuge tube (15 mL, polypropylene), 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 minutes. 0.5 mL

of 1% aqueous NH₄HCO₃ solution was added to the supernatant (~4.5 mL), the solution was separated into an aqueous layer (upper layer, MeOH and H₂O) and DCM-MeOH layer (lower layer).

2.2 HPLC-ICPMS/ESIMSMS analysis of water-soluble arsenic species and arsenolipids

Separation was performed under reversed-phase conditions using a Shodex Asahipak C8P-50 4D column (4.6 × 150 mm, 5 μm particle size). The column effluent was split using a passive splitter with 80% being transferred directly to the ESI-MSMS. The remaining 20% of the split flow was transferred to the ICPMS together with a support flow of water containing 1% formic acid and 20 μg L⁻¹ Ge, In, Te (0.3 mL min⁻¹) introduced through a T-piece after the splitter.

3 RESULTS AND DISCUSSION

3.1 Results

Arsenic species in the aqueous layers were analyzed, and revealed that two arsenosugars were produced by *Nostoc*. The relative proportion of arsenosugars in total aqueous arsenic species in *Nostoc* cells was 0.5%–13% for Oxo-Gly and 0.7%–8% for Oxo-PO₄, and decreased with increasing As(III) exposure.

HPLC/ICPMS analysis of the DCM fraction showed the presence of arsenolipids, and analysis by ESMSMS revealed that the [M + H]⁺ of two main arsenic-containing compounds were 983 and 985. The chromatographic behavior was the same as that reported previously for arsenosugar phospholipids