



Proteomic analysis reveals contrasting stress response to uranium in two nitrogen-fixing *Anabaena* strains, differentially tolerant to uranium



Bandita Panda, Bhakti Basu, Celin Acharya, Hema Rajaram, Shree Kumar Apte*

Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India

ARTICLE INFO

Article history:

Received 30 August 2016

Received in revised form

30 November 2016

Accepted 2 December 2016

Available online 5 December 2016

Keywords:

Uranium exposure

Diazotrophic cyanobacteria

Anabaena 7120

Anabaena L-31

Proteomic response

Photosynthesis

Oxidative stress

ABSTRACT

Two strains of the nitrogen-fixing cyanobacterium *Anabaena*, native to Indian paddy fields, displayed differential sensitivity to exposure to uranyl carbonate at neutral pH. *Anabaena* sp. strain PCC 7120 and *Anabaena* sp. strain L-31 displayed 50% reduction in survival (LD_{50} dose), following 3 h exposure to 75 μ M and 200 μ M uranyl carbonate, respectively. Uranium responsive proteome alterations were visualized by 2D gel electrophoresis, followed by protein identification by MALDI-ToF mass spectrometry. The two strains displayed significant differences in levels of proteins associated with photosynthesis, carbon metabolism, and oxidative stress alleviation, commensurate with their uranium tolerance. Higher uranium tolerance of *Anabaena* sp. strain L-31 could be attributed to sustained photosynthesis and carbon metabolism and superior oxidative stress defense, as compared to the uranium sensitive *Anabaena* sp. strain PCC 7120.

Significance: Uranium responsive proteome modulations in two nitrogen-fixing strains of *Anabaena*, native to Indian paddy fields, revealed that rapid adaptation to better oxidative stress management, and maintenance of metabolic and energy homeostasis underlies superior uranium tolerance of *Anabaena* sp. strain L-31 compared to *Anabaena* sp. strain PCC 7120.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Uranium is known to adversely influence life through its chemical toxicity, rather than its radiological toxicity (Qi et al., 2014). Anthropogenic activities like uranium mining, nuclear fuel production and reprocessing, and waste disposal can lead to migration of uranium to groundwater, leading to contamination of soil and water and metal bioaccumulation (Taylor and Taylor, 2011). Contemporary agriculture utilizes phosphate fertilizers (e.g. superphosphates) and biofertilizers (e.g. diazotrophic cyanobacteria) in paddy fields for enhancing crop productivity. Rock phosphates of sedimentary origin, generally used for the production of phosphate fertilizers, contain 1.0–5.7 Bq g⁻¹ uranium (²³⁸U) and serve as a potential source of natural radionuclide contamination (Barisic et al., 1992). Uranium concentrations ranging from 15.9 to 35.8 mg L⁻¹ have been reported in phosphate fertilizers in India (Lal et al., 1985; Yamazaki and Geraldo, 2003), while in other countries, it ranges from 3.2 to 221 mg L⁻¹ (Yamazaki and

Geraldo, 2003). Environmental contamination with radionuclides like uranium causes serious problems for safer use of agricultural land, including resident microbes therein, thereby posing a serious threat to ecosystem and human health (Taylor and Taylor, 2011). To improve soil and crop productivity, physio-chemical removal of uranium is necessary, but is difficult and rather expensive (Barisic et al., 1992). Photosynthetic, cyanobacteria naturally abound in tropical fields and some of them are common occurrence in metal contaminated environments, which often accumulate and detoxify metal contaminants from soil and water (El-Enany and Issa, 2000; Noraho and Gaur, 1996; Gale and Wixson, 1979; Li et al., 2004; Kanamaru et al., 1994). Two strains chosen for the present study, *Anabaena* sp. strain PCC 7120 is a sequenced strain (<http://genomekazusa.or.jp/Cyanobase/anabaena>) and *Anabaena* strain L-31 is native to Indian paddy fields. These cyanobacteria regularly experience environmental stresses like salinity, desiccation, heat, salinity and heavy metals (Apte, 2001). The genome of *Anabaena* sp. strain PCC 7120 is known to harbour a plethora of genes encoding reactive oxygen species (ROS) mitigating enzymes, to alleviate oxidative stress induced by different stresses, including heavy metals (Banerjee et al., 2013; Acharya and Apte, 2013; Bhargava et al., 2008; Panda et al., 2014; Zhao et al., 2007; Banerjee et al., 2012a,b;

* Corresponding author.

E-mail addresses: aptesk@barc.gov.in, sksmbd@barc.gov.in (S.K. Apte).

Raghavan et al., 2011). Unravelling of the molecular mechanisms involved in heavy metal detoxification in these paddy-field inhabitants will help in establishing cost effective in situ bioremediation strategies for contaminated sites. Proteomic analyses are extensively useful in revealing the molecular mechanisms employed by the microbes to combat various stresses (Bhargava et al., 2008; Panda et al., 2014).

The present study reports proteome modifications in two filamentous, photosynthetic, nitrogen-fixing cyanobacteria, *Anabaena* sp. strain PCC 7120 (hereafter referred to as *Anabaena* 7120) and *Anabaena* sp. strain L-31 (hereafter referred to as *Anabaena* L-31), exposed to sub-lethal (50% growth inhibitory or LD₅₀) concentrations of uranium. A total of 79 proteins from *Anabaena* 7120 and 64 proteins from *Anabaena* L-31 were identified by MALDI mass spectrometry in response to uranium exposure, of which levels of 45 and 27 proteins, respectively were found to be differentially modulated in the two strains. The results provide an insight into the adaptive response of nitrogen-fixing *Anabaena* strains, involved in alleviation of uranium stress.

2. Materials and methods

2.1. Cyanobacterial strains, growth conditions and uranium treatment

Two cyanobacterial strains, namely *Anabaena* PCC 7120 and *Anabaena* L-31 were grown in combined nitrogen free BG-11 medium, pH 7.2 under continuous illumination ($30 \mu\text{E m}^{-2} \text{s}^{-1}$) and aeration (3 L min^{-1}) at $27 \pm 2^\circ\text{C}$ (Mackinney, 1941). Growth was measured in terms of chlorophyll *a* estimated in 90% methanolic extracts at 665.4 nm, as described earlier (Castenholz, 1998). For determination of LD₅₀ for uranium, exponential phase cultures of the two strains were exposed to different concentrations of uranyl carbonate (50–300 μM) prepared as described earlier (Acharya et al., 2009), at a density of $10 \mu\text{g}$ chlorophyll *a* mL^{-1} , and incubated under shaking (120 rpm) and illumination ($30 \mu\text{E m}^{-2} \text{s}^{-1}$) for specified periods of time (1–6 h). Survival following uranium exposure was evaluated by plating $100 \mu\text{L}$ culture aliquots on combined nitrogen free BG-11 agar plates and counting the number of colony forming units (CFU) after 10 days of incubation, under continuous illumination at $27 \pm 2^\circ\text{C}$. Each experiment comprised of three replicates and the observed variation between the experiments was found to be less than 10%.

2.2. Estimation of uranyl binding

Experimental media (BG-11 medium) were allowed to equilibrate for 30 min after addition of LD₅₀ concentration (75 μM and 200 μM for *Anabaena* 7120 and *Anabaena* L31, respectively) of uranyl carbonate [$\text{UO}_2(\text{CO}_3)_2$]²⁻. Experiments were initiated by inoculating an equivalent density ($10 \mu\text{g}$ of chlorophyll *a* mL^{-1}) of both the cyanobacterial strains, separately in the test solutions and incubating them under continuous shaking and illumination for 3 h. Aliquots ($100 \mu\text{L}$) were withdrawn at timed intervals and centrifuged at 13,000 rpm for 3 min. The supernatants (residual uranium) were acidified with 0.01 N HCl to prevent precipitation. The uranium loaded cell pellets (washed with distilled water to remove loosely bound uranium) were digested with 0.2% HCl at room temperature. Both the mineralized fractions were assayed for uranium using arsenazo III method (Savvin, 1961).

2.3. Extraction and separation of proteins by 2-D electrophoresis

Uranium treated (75 μM and 200 μM Uranyl carbonate for *Anabaena* 7120 and *Anabaena* L-31 respectively, for 3 h) cyanobacterial cells (equivalent of $\sim 150 \mu\text{g}$ chl *a*) along with respective

untreated control cells were harvested by centrifugation at 5000 rpm for 5 min and resuspended in lysis buffer [1 mM Tris-HCl, pH 8 containing 1 mM phenylmethylsulfonyl fluoride (PMSF)]. The cells were lysed by freeze and thaw method (Panda et al., 2014) and proteins extracted from each strain by centrifugation at 14,000 rpm for 30 min at 4°C . The protein content was estimated using a Lowry protein estimation kit (Sigma, India). The protein extracts were subjected to simultaneous DNase I and RNase I ($10 \mu\text{g mL}^{-1}$ each) for 1 h on ice in each case. For protein separation, proteins ($\sim 1 \text{ mg}$) from each strain were concentrated under vacuum to $10 \mu\text{L}$ and then solubilized in $80 \mu\text{L}$ of rehydration buffer {8 M urea, 1 M thiourea, 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-1-propanesulfonate (CHAPS), 150 mM dithiothreitol (DTT), 2% immobilized pH gradient (IPG) buffer, traces of Bromophenol Blue} for 1 h at room temperature and loaded on the IPG strips, nonlinear pH 3–10, 17 cm (Bio-Rad, India) by cup-loading method following the protocol as described earlier (Panda et al., 2014). The iso-electric focusing (IEF) was performed with the Protean Isoelectric Focusing Cell (Bio-Rad, India) at 20°C and the 2nd dimensional resolution was performed by 14% SDS-PAGE. Each experiment was repeated three times in each strain resulting in 3 biological replicates each.

2.4. Gel imaging and spot analysis

Gels were imaged by Dyversity-6 gel imager (Syngene, UK) using Gene Snap software (Syngene, UK). PD-Quest (version 8.1.0, Bio-Rad) was used to generate a first level match set from three biological replicates of 2D gels with a minimum correlation coefficient value of 0.6. Spot detection and matching between replicate gels were done in automatic detection mode, followed by manual editing to exclude those spots that were not present on all replicate gels. The spot densities were normalized using local regression method. Statistical analysis was performed by independent Student's *t*-test and the protein spots with *p*-values less than 0.05 were considered as significantly altered between control and treated sample of three strains.

Eighty three protein spots from *Anabaena* 7120 and 64 protein spots from *Anabaena* L-31 were excised from the gel, followed by repeated washing with 50 mM $\text{NH}_4\text{HCO}_3/\text{ACN}$, reduction with DTT (Sigma, India), alkylation with 55 mM iodoacetamide (Sigma, India). A standard protocol for in-gel trypsin digestion and elution of oligopeptides was used (Panda et al., 2014). Eluted peptides were vacuum concentrated to a final volume of $5 \mu\text{L}$, if necessary.

2.5. Mass spectrometry and protein identification

The eluted polypeptides were co-crystallized with α -cyano-4-hydroxycinnamic acid (CHCA) matrix [5 mg mL^{-1} in 0.1% trifluoroacetic acid (TFA) and 30% acetonitrile (ACN)] on a 384-well ground steel target plate (Bruker Daltonics, Germany). The Matrix-assisted laser desorption/ionization-Time of Flight (MALDI ToF/ToF) UltraFlexIII mass spectrometer was externally calibrated using Peptide calibration mix I (Bruker Daltonics, Germany) as per the manufacturer's protocol. The analysis was carried out in positive ion reflector mode and the mass spectra were acquired with standard ToF-MS protocol in the mass range of 600–4500 Da. Spectra were acquired using FlexAnalysis software 3.0 (Bruker Daltonics) as detailed earlier (Panda et al., 2014). The mass spectra were imported into the database search engine (BioTools v3.1 connected to Mascot, Version 2.2.04, Matrix Science). Settings of Mascot search from NCBI nonredundant database (released Jan 2012 or later, at least 17910093 entries actually searched) or SwissProt database (released Jan 2012 or later, at least 539616 entries actually searched) chosen for identification were: number of missed cleavages permitted 1 or 2; fixed modifications such as carbamidomethyl on cysteine; variable modification of oxidation

Download English Version:

<https://daneshyari.com/en/article/5764353>

Download Persian Version:

<https://daneshyari.com/article/5764353>

[Daneshyari.com](https://daneshyari.com)