JOURNAL OF PROTEOMICS 75 (2012) 921–937

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

Proteomics combines morphological, physiological and biochemical attributes to unravel the survival strategy of *Anabaena* sp. PCC7120 under arsenic stress

Sarita Pandey[a], *Rashmi Rai*[b], *Lal Chand Rai*[a,*]

[a]*Molecular Biology Section, Laboratory of Algal Biology, Center of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India*
[b]*Laboratory of Morphogenesis, Center of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India*

ARTICLE INFO

Article history:
Received 2 July 2011
Accepted 17 October 2011
Available online 25 October 2011

Keywords:
Arsenic
Anabaena sp. PCC7120
2-DE
MALDI-TOF/MS
RT-PCR
Western blotting

ABSTRACT

Proteomics in conjunction with morphological, physiological and biochemical variables has been employed for the first time to unravel survival strategies of the diazotrophic cyanobacterium *Anabaena* sp. PCC7120 under Arsenic (As) stress. Significant reduction in growth, carbon fixation, nitrogenase activity and chlorophyll content after 1 day (1 d) and recovery after 15 days (15 d) of As exposure indicates the acclimation of the test organism against As stress. The formation of akinete like structures is a novel observation never reported before in *Anabaena* sp. PCC7120. Proteomic characterization using 2-DE showed average 537, 422 and 439 spots in control, 1 and 15 d treatment respectively. MALDI-TOF and LC-MS of As-treated *Anabaena* revealed a total of 45 differentially expressed proteins, of which 13 were novel (hypothetical) ones. Down-regulation of phosphoglycerate kinase (PGK), fructose bisphosphate aldolase II (FBA II), fructose 1,6 bisphosphatase (FBPase), transketolase (TK), and ATP synthase on day 1 and their significant recovery on the 15th day presumably maintained the glycolysis, pentose phosphate pathway (PPP) and turnover rate of Calvin cycle, hence survival of the test organism. Up-regulation of catalase (CAT), peroxiredoxin (Prx), thioredoxin (Trx) and oxidoreductase appears to protect the cells from oxidative stress. Appreciable induction in phytochelatin content (2.4 fold), GST activity (2.3 fold), and transcripts of phytochelatin synthase (5.0 fold), arsenate reductase (8.5 fold) and arsenite efflux genes — *asr1102* (5.0 fold), *alr1097* (4.7 fold) reiterates their role in As sequestration and shielding of the organism from As toxicity. While up-regulated metabolic and antioxidative defense proteins, phytochelatin and GST work synchronously, the *ars* genes play a central role in detoxification and survival of *Anabaena* under As stress. The proposed hypothetical model explains the interaction of metabolic proteins associated with the survival of *Anabaena* sp. PCC7120 under As stress.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

India is one of the largest countries of the world requiring a huge amount of food production in limited agricultural land to feed its gigantic population. According to the estimates, the total rice production in India is about 148,260,000 metric tons, bringing the country second only to China (193,354,175 metric tons) in world rice production [1]. Unfortunately, abiot-

Abbreviations: PGK, phosphoglycerate kinase; FBA II, fructose bisphosphate aldolase II; FBPase, fructose 1,6 bisphosphatase; TK, transketolase; PPP, pentose phosphate pathway; CAT, catalase; Prx, peroxiredoxin; Trx, thioredoxin; *pcs*, phytochelatin synthase; GST, glutathione S-transferase

* *Corresponding author*. Tel.: +91 542 6701110; fax: +91 542 2368174.
E-mail addresses: lcrbhu15@gmail.com, lcrai@bhu.ac.in (L.C. Rai).

1874-3919/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jprot.2011.10.011

ic stresses like salinity, drought, heat, pesticides, and heavy metals are negatively affecting crop productivity. Nevertheless, the increasing groundwater contamination by arsenic and its use for irrigation as well as excessive use of chemical fertilizers and metal-containing pesticides are not only jeopardizing the survival of microbial communities including cyanobacteria inhabiting therein but leaving the soil still worst for cultivation especially in South East Asian countries [2,3].

Cyanobacteria, the photosynthetic prokaryotes, contribute substantially to the nitrogen economy and constitute a prominent component of microbial population in wetland soils, especially rice paddy fields [4] and play a vital role in the maintenance of soil fertility and rice productivity. The long evolutionary history, 3.5 billion years, of cyanobacteria is a testimony to their adaptive capability against environmental vagaries thereby contemplating them as an excellent model to study stress responses. Although a large number of cyanobacteria including species of *Anabaena* are known to fix nitrogen, the test cyanobacterium *Anabaena* sp. PCC7120 (hereafter *Anabaena*), is interesting because of the availability of its full genome sequence and tolerance to environmental stresses [5].

Cyanobacterial adaptation to stress is coupled with profound changes in proteome repertoire. Since proteins are directly involved in stress responses, proteomic studies can unravel the possible relationships between protein abundance and stress acclimation. Over the past years, the proteomic approach has been applied to analyze the proteins involved in stress responses in cyanobacteria, such as Cu [6], salinity [7,8], heat and UV-B [9–11], butachlor [12], and Fe starvation [13]. However, As-induced proteomic changes in cyanobacteria have remained unexplored as yet. Information on As toxicity in cyanobacteria are confined to phosphate arsenate interaction in *Synechococcus leopoliensis*, *Synechococcus* sp. [14,15], *Anabaena variabilis* [16], *Anabaena* PCC7120 [17], bioaccumulation of As in *Phormidium* sp., *Phormidium laminosum* [18–20], and antioxidative enzymes of *Anabaena doliolum* [21], *Phormidium laminosum* [20], and the role of arsenic resistance genes (*ars* operon) in *Synechocystis* [22].

Arsenic adversely affects the metabolic processes and leads to (i) oxidative stress through ROS generation thereby damaging proteins, lipids and nucleic acids [23,24], (ii) inhibition of RubisCO [25], chlorophyll biosynthesis, photosynthetic pigments [21,26], photosynthesis [27], and (iii) inhibition of ATP [28] by uncoupling phosphorylation. Arsenic stressed organisms have been reported to adopt to one of the following strategies: (i) competitive inhibition of arsenate uptake by phosphate [15], (ii) over induction of GSTs [29], (iii) biotransformation of arsenic species [30], (iv) GSH and phytochelatin (PC) based sequestration of As [31–33], and (v) reduction of arsenate into arsenite and its efflux from the cell through *arsC* and *arsB* genes respectively [34].

The information available on the *ars* genes of bacteria [34], yeast [35] and plants [36,37] are interesting but quite divergent. The *ars* genes in bacteria are in the form of an *ars* operon present both on the chromosome as well as plasmid. The *ars* operon is constituted by *arsR*, *-B*, and *-C* encoding a *trans*-acting repressor, an arsenite efflux protein and an arsenate reductase respectively. Two additional genes *arsA* and *arsD* encoding an arsenite-stimulated ATPase and a metalloid-responsive transcriptional repressor are also known. Notwithstanding above, the *ars* genes of *Synechocystis* [22] are in the form of *arsBHC* operon containing three genes: *arsB* (arsenite efflux protein), *arsH* unknown protein and *arsC* (arsenate reductase) regulated by the transcriptional repressor *arsR*. In silico analysis of the *Anabaena* genome depicted the presence of a cluster of 4 genes (http://genome.kazusa.or.jp/cyanobase/Anabaena): *asr1102* (homologue of *arsB*), *all1103* (transcriptional regulator *arsR*), *alr1104* (hypothetical protein), and *alr1105* (arsenate reductase); while three genes are arranged in one orientation, *all1103* is divergently arranged, hence their operonic structure is different from other *ars* operons. In addition to above an *arsB* homologue *alr1097* (multidrug efflux protein) conceivably involved in As stress management is also present upstream to *asr1102*. It is worth mentioning that apart from *alr1103*, 8 transcriptional regulators (*alr2766*, *alr1867*, *alr1044*, *alr0831*, *all5056*, *all3903*, *all3743*, *and all7621*) belonging to the ArsR family having 50.6–67.3% similarity with the *arsR* (*sll1957*) of *Synechocystis* are also present in the genome of *Anabaena*. Furthermore, the catalytic residues of arsenate reductase from *Anabaena* (CKHNSRRS) differ in respect to some amino acids from *Staphylococcus* (CTGNSCRS) and *Synechocystis* (CKRNSCRS).

In view of the above the major gaps identified in relation to cyanobacteria and As stress include: (i) As toxicity has never been addressed in N_2-fixing cyanobacteria employing proteomics along with morphological, physiological, and biochemical variables, (ii) complete lack of data on the role of *ars* and other genes like *pcs*, thioredoxin, peroxiredoxin, *gst* of *Anabaena* in As toxicity management. The present study therefore combines proteomics in tandem with morphological, physiological, and biochemical attributes, and selected stress responsive genes to decipher the mechanism of As toxicity in diazotrophic cyanobacteria.

2. Materials and methods

2.1. Experimental setup and growth measurement

Anabaena was cultured in BG-11 medium [38] buffered with HEPES (0.5 g L^{-1}) at 24±2 °C under 72 μmol photon m^{-2} s^{-1} PAR (photosynthetically active radiation) with a photoperiod of 14:10 h (light:dark) at pH 7.5. A 40 mM of As ($Na_2HAsO_4{\cdot}7H_2O$) selected for the experiment was LC_{50} concentration, as determined by the plate colony count method of Rai and Raizada [39]. Time durations selected for this work were 1 d (short term) and 15 d (long term). Exponentially growing cells of *Anabaena* were treated with 40 mM As and the growth estimated by measuring the OD (optical density) of the culture at 750 nm in a UV–VIS spectrophotometer (Systronics, India) on every third day up to the 15th day. Chlorophyll a and phycocyanin were measured as per the method of Bennett and Bogorad [40] by taking the absorbance at 663 and 645 nm respectively. Microphotographs of periodic-schiff reagent stained [41] and unstained cells were taken by phase contrast microscope (Nikon E800). All the experiments were done with three biological replicates.

2.2. Arsenic concentration measurement

Oven-dried cyanobacterial pellet (50 mg) was digested in 3:1 HNO_3 and H_2O_2 [42]. Total As was estimated by atomic

Download English Version:

https://daneshyari.com/en/article/10556082

Download Persian Version:

https://daneshyari.com/article/10556082

Daneshyari.com