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Nickel and Arsenite Responsive Proteomic Alterations in Cyanobacterium *Anabaena* PCC7120

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Abstract: **Ecological adaptation of organisms to environmental stresses including heavy metals denotes a novel example of evolution under extreme selection pressure. Cyanobacteria represent the largest and most diverse group of prokaryotes, capable of performing oxygenic photosynthesis and are frequently found in environments contaminated with heavy metals. Quite a lot of proteomic studies have investigated the effects of metals such as Cu, Co, Cd, and As(V) etc. on diazotrophic cyanobacteria. However, proteomic studies for the identification of proteins modulated Ni and arsenite [As(III)] have not been carried out. In the present work, we have analyzed the proteomic pattern alterations of the cyanobacterium *Anabaena* PCC7120 in response to Ni and As(III) in order to identify the metabolic processes affected by these metals. We found that some proteins are commonly regulated in response to the different metal, including ribulose1,5-bisphosphate carboxylase, chaperones and antioxidative defence proteins, whereas others such as specific efflux proteins, were specifically induced by each metal. Our results demonstrated that arsenite appears to be more toxic than nickel.**

Index Terms: **Cyanobacteria, *Anabaena* PCC7120, Nickel, Arsenite, 2-DE, MALDI-TOF/MS**

I. Introduction

Cyanobacteria, also known as blue green algae, are prokaryotic organisms which evolved 300 million year ago and have been found to be a major organism in contributing in rise of atmospheric oxygen (Rasmussen et al, 2008). Fossil records of 3,450 million year old from Warrawoona sedimentary rock of north-western Australia explain cyanobacteria were among the pioneer organisms of the early earth and constituting the largest prokaryotic group on the earth. They are oxygenic photoautotrophic organism which has the ability to produce oxygen which made the environment to evolve from an anaerobic to an aerobic one (Lochte & Turley, 1988). Due to their ability of photosynthetic growth in the presence of oxygen, together with having water as their electron donor for CO2 reduction, enables cyanobacteria to occur in a wide range of ecological niches (Whitton, 1992). They are also found to fix atmospheric nitrogen thus acting as a biofertilizer especially in paddy field like the association of *Anabaena*-azolla (Venkataraman, 1972). Hence, they constitute to be one of the major organisms of anthropogenic importance but unfortunately, they are exposed to various abiotic stresses thus decreasing their significant contribution in ecosystem.

Cyanobacteria are exposed to various stresses like UV, salinity, temperature, herbicides, etc., but in addition to the above they are also frequently challenged by heavy metals, such as aluminium (Al), arsenic (As), cadmium (Cd), caesium (Cs), chromate (Cr), mercury (Hg), lead (Pb), or uranium (U), which play no significant role (Tchounwou et al, 2012). Various anthropogenic activities have led to the accumulation of such heavy metals in the environment resulting into altered biogeochemistry (Bhagat et al, 2016). The accumulation of heavy metal in soils either due to anthropogenic activity (mining, burning fossil fuels, etc.) or by natural sources (volcanoes or forest fires) has led to grave environmental contamination due to their persistence in the environment (Song et al, 2014).

Although few metals hold prime importance in biotic component as they play key role in their various basic life processes but their elevated concentration causes undesirable effects (Waldron et al, 2010). One of the major stresses in paddy fields is heavy metals stress which is due to irrigation of fields with polluted water thus leading to accumulation of heavy metal possessing detrimental effect on soil. Cyanobacteria are major component of microbial population in wet land soils, especially in paddy fields, as they play key role in providing fixed nitrogen thus enhancing soil fertility. Hence, cyanobacteria are exposed to heavy metals in paddy fields (Yadav et al, 2015).

Nickel (Ni) is one of the metals that function in cellular physiology of living organism (Poonkothai and Vijaywathi,

2012) but some anthropogenic activities that caused an elevated level of Ni into the environment leading to its detrimental effects are chemical industries (planting, metal finishing, pigment production and cement manufacturing), disposal of NiCd batteries, mining, energy supplying stations (Nnorom and Osibanjo, 2009).

Apart from nickel, the increasing groundwater contamination by arsenic and its use for irrigation as well as excessive use of chemical fertilizers and metal-containing pesticides are effecting survival of microbial communities including cyanobacteria inhabiting therein but it is also making the soil worst for cultivation especially in South East Asian countries (Mehrag, 2004; Dhankher, 2005).

The toxic effect of heavy metals is related to the production of reactive oxygen species. Cyanobacteria respond to heavy metals by altering several antioxidant enzyme activities such as superoxide dismutase, catalase, glutathione peroxidase, ascorbate peroxidase, and the synthesis of low molecular weight compounds such as carotenoids and glutathione (Rosen, 1999). In our previous study (Prajapati et al, 2018), oxidative stress and antioxidant response in two *Anabaena* species (*Anabaena* PCC7120 and *Anabaena* doliolum) exposed to nickel and arsenite stress was investigated which suggested the enhance activities of antioxidant enzymes and the decrease in MDA contents in the cells as an adaptive response to metal stress. Cyanobacterial adaptation to stress is coupled with profound changes in proteome.

High concentrations of metal ions are known to promote a variety of damages at the molecular level, including mostly oxidative stress, or misfolding polypeptide chains (Briggs et al, 1990; Brostrom & Brostrom, 1998). Since proteins are directly involved in stress responses, proteomic studies can unravel the possible relationships between protein abundance and stress acclimation (Pandey et a., 2012). In continuation to our previous study the present paper investigated the response of *Anabaena* PCC7120 at the proteomics level to study the up regulation and down regulation of proteins, when exposed to nickel and arsenic stress. Further, *Anabaena* PCC7120 was selected as an experimental organism because of the availability of its full genome sequence and tolerance to environmental stresses.

II. Materials and Methods

A. Organisms and growth conditions

Anabaena sp. PCC 7120 was grown photoautotrophically at 25 ± 2°C in BG-11 medium buffered with HEPES/NaOH under daylight fluorescent tubes emitting 72 µmol photon m^{-2} s^{-1} PAR (photosynthetically active radiation) light intensity with a photoperiod of 14:10 h at pH 7.5. The cultures were shaken by hand 2–3 times daily for aeration.

B. Experimental design and treatment

The average IC50 for *Anabaena* sp. PCC 7120 for Ni^{2+} is 15 µM and for arsenite 17 mM (Prajapati et al, 2017). A 100mM and 100 µM stock solution of Sodium meta-arsenite and nickel chloride respectively were sterilized by passing through the Millipore membrane filter (0.22 µM) and calculated amount were added directly into the sterilized medium to achieve the desired concentration and working standards were obtained by further dilutions. Exponentially growing cells of *Anabaena* PCC 7120 was treated with respective LC50 doses and collected at one time points (1 day). Untreated cells were used as control. Three biological replicates of each were used for comparing their proteomes at control and 1day of treatment.

C. Extraction, quantification and 2-DE separation of proteins

Anabaena cells were harvested by centrifugation (10,000× g, 10 min.) followed by resuspension in 2 ml extraction buffer containing 10 mM Tris-Cl (pH 8.0), 1.5 mM $MgCl_2$ and 10 mM KCl in the presence of 10 µM PMSF (Sigma). The suspension was ground in liquid nitrogen followed by centrifugation at 12,000× g for 1 h. The supernatant containing cytosolic protein (500 µl) were precipitated and solubilized in a buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 40 mM DTT and 1.0% IPG buffer (4–7) and the protein concentration measured using Bradford method (Bradford, 1976). Solubilization protein containing ~250 µg protein samples were incubated with dry IPG strips (pH 4–7;13 cm; GE Healthcare, USA) at 20 °C for 12–16 h at room temperature. The focusing was performed at 20 °C with an in 7 steps: linear 30 V for 00.30 h, 150 V for2:00 h, 300 V for 00:40 h, 500 V for 4 h, gradient 1000 V for 1 h, gradient 8000 V for 2 h and finally 8000 V for 13:00 h. After IEF IPG strips were equilibrated by first incubating in an equilibration solution (6 M urea, 30% glycerol, 2% SDS, 50 mM Tris–HCl, pH 8.8, and 1% DTT and a trace amount of bromophenol blue) for 15 min followed by incubation in in the same equilibration solution containing 2.5% iodoacetamide instead of 1%DTT for 15 min. The strip was placed on the top of 12.0% SDS-PAGE and electrophoresis was carried out at 10 mA/gel for 30 min followed by 25 mA/gel for 5 h. The gels were stained with CBB R-250.

D. 2-DE gel analysis, protein quantification and identification

2-DE gel image analysis software PDQuest™Basic version 8.0.1(Bio-Rad) was used for spot quantification between Ni and As(III) treated and untreated control as spot volumes (intensity × mm2). Protein spots having statistically significant ($p<0.05$) and reproducible changes with an abundance ratio of at least 1.4 fold were taken as differentially expressed proteins and subjected to MALDI-TOF MS/MS analysis. Samples for MALDI-TOF analysis were prepared as per Singh et al, 2015. MS analysis was followed by homology search using MASCOT. Data acquisition and analysis were performed using flex control and flex analysis/BioTools version 3.2 software, respectively (Bruker Daltonics). The protein databases employed were, NCBInr (The National Center for Biotechnology Information non-redundant) and Swiss-Prot. The peptide mass fingerprinting (PMF) and

MS/MS ion search were performed with the following MASCOT settings: taxonomy as bacteria; peptide mass tolerance of ±50 to ± 100 ppm for peptide mass fingerprinting and ±1.2 Da for MS/MS, monoisotopic mass, alkylation of cysteine by carbamidomethylation as a fixed modification and oxidation of methionine as a variable modification. The peptide sequences with the highest scores of each spot were validated by BLAST P analysis.

III. Result and Discussion

A. MALDI-TOF MS and LC-MS identification and classification of nickel and arsenite responsive proteins

A total of 102, 84 and 79 protein spots were analysed by PDQuest software in 2-DE gels of control, Ni^{2+} and arsenite (AsIII) treatments, respectively (Fig. 1). These proteins were further classified into 5 functional categories as per cyanobase (http://genome.microbedb.jp/cyanobase/*Anabaena*): (1) energy metabolism (photosynthesis, carbon fixation and respiration); (2) ROS scavenging and defense; (3) protein synthesis and folding (4) nucleotide synthesis; (5) hypothetical proteins (Table I and II). Identified proteins were divided into two major groups on the basis of their expression pattern. While the first group comprising of 19 proteins depicted common upregulation, the second group with 6 proteins was downregulated under Ni and arsenite (AsIII) (Table III). Interestingly however, few proteins were upregulated in Ni, but downregulated in arsenite (AsIII) and vice versa. Also few proteins were specifically expressed in one stress and absent from the gel of other stress. The above results represent a significant difference in the response of *Anabaena* to two stresses and call for further investigation.

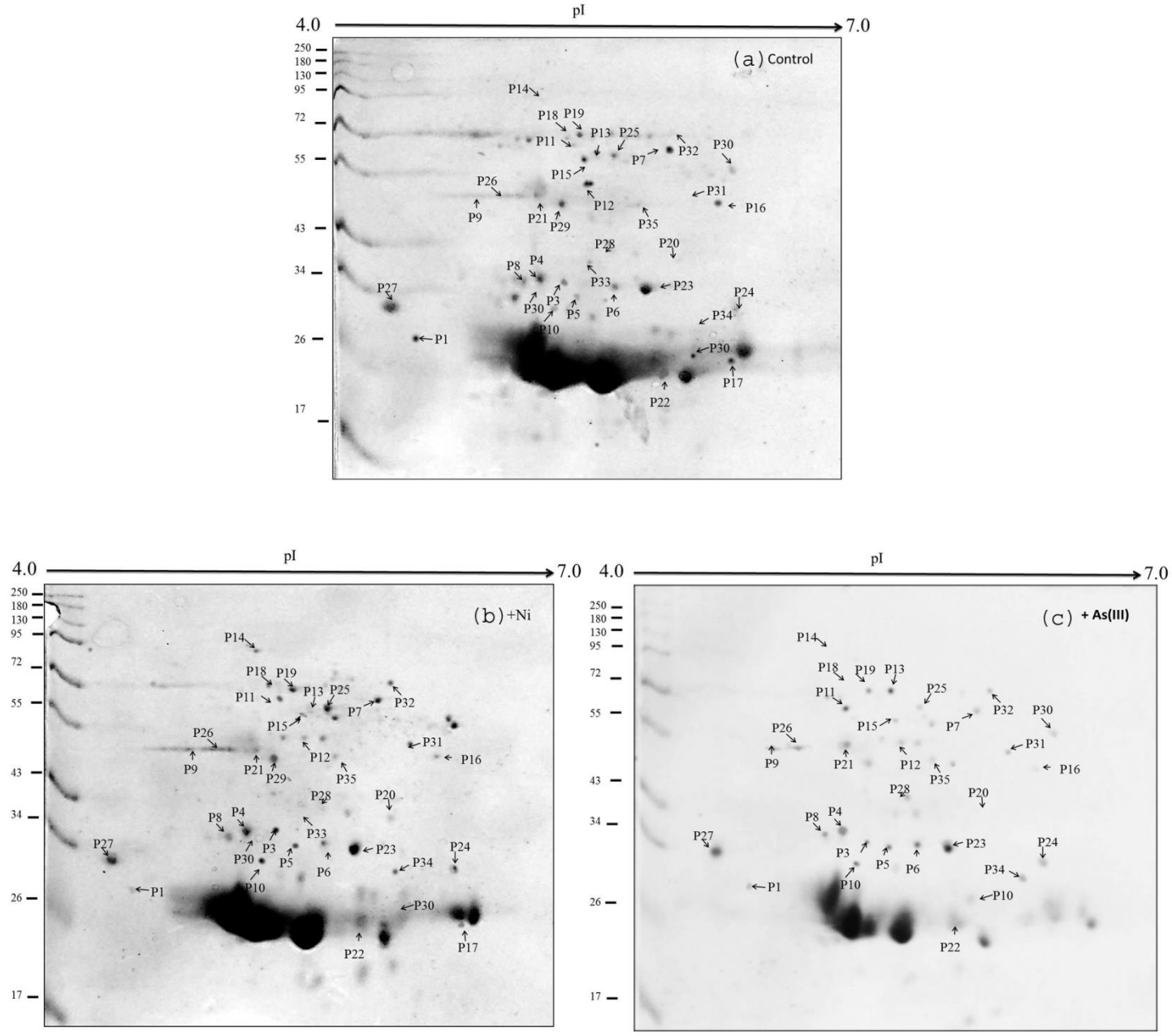
B. Proteomic response of Anabaena PCC 7120 to Ni^{2+} stress

Ni is an essential metal for the catalytic activity of several enzymes however known to inhibit processes such as respiration and photosynthesis at high concentrations and considered as a highly toxic metal. For cell survival, maintenance of a precise homeostasis through sensing and transport systems holds extreme importance. Uptake of Ni in microbial organisms is known to occur through non-specific transport systems and high affinity specific systems such as multicomponent ABC (ATP-binding cassette) transport systems (Navarro et al, 1993) and one-component permeases (Eitinger & Friedrich, 1991; Mobley et al, 1995; Fu et al, 1994). Apart from them, export systems are likewise important for metal homeostasis, for example, the Ncc (nickel, cobalt, cadmium) system membrane protein complexes are involved in Ni efflux (Grass et al, 2001; Collard et al, 1993; Liesegang et al, 1993; Schmidt & Schlegel, 1994).

In present study, we have explored the detailed protein variations that occur in *Anabaena* PCC7120 in the presence of 15 μM $NiCl_2$, at inhibitory concentration. The results obtained showed that excess Ni mainly targeted proteins related to photosynthesis and carbon metabolism (Fig. 1a and Table I), which is similar with other studies conducted on heavy metal effects in plants and microorganisms (Aggarwal et al, 2012; Cox & Saito, 2013; Singh et al, 2015). Photosynthesis relies on the cooperation of light (photosynthetic electron transport) and dark reactions (CO_2 fixation). Rubisco large subunit (rbcL), first key enzyme of Calvin cycle displayed downregulation rendering carbon fixation as one of the most pretentious pathways under nickel stress in *Anabaena* PCC7120. Several other proteins related to photosynthesis showed downregulation such as, phycobilisome core component (the major antenna system of most cyanobacteria) and NADH dehydrogenase chain J. Enzymes active in catalyzing key reaction in energy producing metabolic pathways were also downregulated. Fructose 1,6 – bisphosphatase increases the synthesis of fructose 6-phosphate and glucose, downregulation of fructose 1,6 –bisphosphatase also indicates inhibition of carbon fixation. Phosphoglycerate kinase, a monomeric two domain enzyme involved in the Calvin cycle and energy conserving phase of glycolysis also displayed downregulation. Another protein uroporphyrinogen decarboxylase, member of the tetrapyrrole biosynthetic pathway required for chlorophyll biosynthesis was down-accumulated suggesting inhibitory effect on chlorophyll biosynthesis. Apart from these proteins several other proteins such as glutamate ammonia ligase and nutrient stress induced DNA binding protein were also down-accumulated.

In contrast to above mentioned proteins, ATP synthase registered up-accumulation which might be required for enhanced ATP synthesis required for energy and maintenance of the Calvin cycle which is similar to previous studies (Pandey et al, 2012). Enhanced expression of antioxidative defense system proteins SOD-B, Fe-SOD, peroxiredoxin and Mn containing catalase suggested adaptive strategy of organism to atypical condition. SODs are involved in superoxide dismutation whereas Mn-catalase (MCAT) associated in hydrogen peroxide detoxification prevent the production of hydroxide radicals. The cells maintained up-accumulation of these proteins to impede overproduction of corresponding ROS in organism.

Nine proteins involved in transcription, translation, chaperone activity and amino acid metabolism showed upregulation. Among the molecular chaperons, chaperonin GroEL and heat shock protein, class J displayed many fold increase under nickel treatment. PPIase B identified as all4287 is responsible for catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides and accelerate folding of proteins and act as molecular chaperones (Rai et al, 2014). Proteins involved in ribosome assembly, post transcriptional processing and translation - 30sRPs1, EFTs and EFTu demonstrated appreciable increase under nickel stress. Accumulation of chaperons is indicative of accumulation of unfolded and non-functional proteins (Cox & Saito, 2013; Lee et al, 2008) under nickel stress, a general increase in the proteins of transcription and translation possibly compensates the denatured proteins and helps the synthesis of new stress proteins. Oxidoreductase (alr5182), a class of enzyme that catalyzes oxidoreduction reactions demonstrated many fold increase which suggests a notable alteration in redox status in response to nickel accumulation. Also nickel induced noteworthy increase in abundance of ATP



dependent Clp protease depicts its strategy to cope with misfolded and/or damaged peptides.

Among six hypothetical proteins, all4050 (PRCH domain containing protein), alr0803 (a signal transduction histidine kinase homologue), all3324 (beta lactamase), all5315 (PRCH domain containing protein), alr0765 (CBS domain containing membrane protein) and alr0946, four proteins all4050, alr0803, all3324 and all5315 displayed up-accumulation under nickel declaring them essential proteins for the system whereas alr0765 and alr0946 displayed decrease in expression.

C. Proteomic response of Anabaena PCC 7120 to Arsenite (AsIII) stress

As (III) reacts with functional groups of cysteine and imidazolium nitrogens of histidine residues (Rosen, 2002) inactivating a host of enzymes the most important being pyruvate dehydrogenase (Schmoger et al, 2000), glutathione reductase, pyruvate oxidase, choline oxidase, transaminase etc. (Santos et al, 2006). However, information on the metabolic processes responsive to high concentrations of As (III) is still limited. In this study, we analysed proteome of *Anabaena* PCC7120 in response to As (III) and compared to the control condition (without As (III)). The results revealed that, as

Table I- Proteins differentially expressed by *Anabaena* PCC7120 in response to 15 μM NiCl2, identified by mass spectrometry.

Protein description	**Spot no.**	**Gene ID**	**Fold-change**
Energy metabolism (photosynthesis, carbon fixation and respiration)			

ATP Synthase *B* subunit (atpB gene product)	11	all5039	2.0
Phycobilisome core component	14	all2327	-1.6
Rubisco large subunit	7	alr1524	-1.5
Ferridoxin NADP reductase	2	all4121	1.8
NADH dehydrogenase chain J	1	all3840	-1.7
Fructose 1,6 –bisphosphatase	12	alr1041	-2.0
Phosphoglycerate kinase	15	all4131	-1.6
Glyceraldehyde-3- phosphate dehydrogenase	16	all5062	-1.9
Uroporphyrinogen decarboxylase	17	all3909	-1.8
Glutamate ammonia ligase	13	alr2328	-1.6
Inorganic pyrophosphatase	4	all3570	1.8
ROS scavenging and defense			
SOD-B,Fe-SOD	5,6	alr2938	1.9
Mn containing catalase	3	alr3090	2.2
Oxidoreductase	35	alr5182	1.7
Peroxiredoxin	8	alr4641	1.8
Nutrient stress induced DNA binding protein	10	alr3808	2.1
Protein synthesis and folding			
30S ribosomal protein S1	9	all0136	1.8
Translation elongation factor EF-Ts	21	all4791	1.6
Translation elongation factor EF-Tu	25	all4337	1.6
ATP dependant clp protease	28	all4358	2.0
ATP dependant clp protease	23	all4357	1.5
Chaperonin GroEL	19	alr3662	2.2
Chaperonin GroEL	18	alr1896	1.7
Heat shock Protein ,classJ	22	alr1809	1.5
Peptidyl prolyl cis trans isomerase B	20	all4287	1.9
Nucleotide Synthesis			
Polyribonucleotide nucleotidyl transferase	24	all4396	1.6
Hypothetical			
Hypothetical	26,27	all4050	2.0
Hypothetical	31,32	alr0803	1.8
Hypothetical	34	all3324	2.1
Hypothetical	29	all5315	2.4
Hypothetical	30	alr0765	-1.5
Hypothetical	33	alr0946	-1.7

Table II-Proteins differentially expressed by *Anabaena* PCC7120 in response to 17 mM NaAsO2, identified by mass spectrometry.

Protein description	**Spot no.**	**Gene ID**	**Fold-change**
Energy metabolism (photosynthesis, carbon fixation and respiration)			
ATP Synthase *B* subunit (atpB gene product)	11	all5039	2.2
Phycobilisome core component	14	all2327	-1.8
Rubisco large subunit	7	alr1524	-1.6
Ferridoxin NADP reductase	2	all4121	-1.6
NADH dehydrogenase chain J	1	all3840	-1.8
Fructose 1,6 –bisphosphatase	12	alr1041	-2.2
Phosphoglycerate kinase	15	all4131	-1.9
Glyceraldehyde-3- phosphate dehydrogenase	16	all5062	-2.5
Glutamate ammonia ligase	13	alr2328	-1.7
Inorganic pyrophosphatase	4	all3570	1.6
ROS scavenging and defense			
SOD-B,Fe-SOD	5,6	alr2938	1.6
Mn containing catalase	3	alr3090	1.7
Oxidoreductase	35	alr5182	1.5
Peroxiredoxin	8	alr4641	1.9
Nutrient stress induced DNA binding protein	10	alr3808	-1.6
Protein synthesis and folding			
30S ribosomal protein S1	9	all0136	1.9
Translation elongation factor EF-Ts	21	all4791	1.6
Translation elongation factor EF-Tu	25	all4337	1.7
ATP dependant clp protease	28	all4358	1.7
ATP dependant clp protease	23	all4357	1.5
Chaperonin GroEL	19	alr3662	1.8
Chaperonin GroEL	18	alr1896	2.1
Heat shock Protein ,classJ	22	alr1809	2.2
Peptidyl prolyl cis trans isomerase B	20	all4287	1.8
Nucleotide Synthesis			
Polyribonucleotide nucleotidyl transferase	24	all4396	1.7
Hypothetical			
Hypothetical	26,27	all4050	1.8
Hypothetical	31,32	alr0803	1.7
Hypothetical	34	all3324	-1.6
Hypothetical	30	alr0893	-1.7
Hypothetical	33	alr1097	1.9

in the case of Ni stress, several proteins were down-regulated in the presence of As (III) (Table II). Several enzymes involved in photosynthesis and carbon metabolism showed reduced intensity including Rubisco large subunit, fructose 1,6 –bisphosphatase, phosphoglycerate kinase, glyceraldehyde-3- phosphate dehydrogenase, phycobilisome core component, NADH dehydrogenase chain J, glutamate ammonia ligase and nutrient

stress induced DNA binding protein. It is possible that the stress caused by the presence of this metal lead to a reduction in the central carbon metabolism as a protection mechanism.

As reported for Ni, following proteins demonstrated downregulation- 1) ATP Synthase B subunit (atpB gene product) related to energy metabolism, 2) SOD-B, Fe-SOD, Mn containing catalase, oxidoreductase related to ROS scavenging and defense, and 3) 30S ribosomal protein S1, translation elongation factor EF-Ts, translation elongation factor EF-Tu, polyribonucleotide nucleotidyl transferase, ATP dependant clp protease, chaperonin GroEL, heat shock protein classJ, ATP dependant clp protease, peptidyl prolyl cis trans isomerase B related to protein synthesis and folding family.

Proteins from hypothetical category which were differentially expressed includes all4050 (PRCH domain containing protein), alr0803 (a signal transduction histidine kinase homologue), all3324 (beta lactamase) alr0893 (Peptidase C56, PfpI), alr1097 (arsenite efflux genes). Among these hypothetical genes, all4050, alr0803 and alr1097 demonstrated upregulation however all3324 and alr0893 were down-accumulated. Interestingly hypothetical protein alr1097 was up-accumulated which shows 82% similarity (http://www.ncbi.nlm.nih.gov/BLAST/) to the known arsenite efflux genes of Synechocystis PCC6803. Up-accumulation of alr1097 suggests that detoxification of arsenite was brought about by efflux by the above putative efflux genes thus relieving the test organism from the As (III) stress. It would be interesting to further explore the induction and the specificity of these hypothetical proteins in order to understand their role in Ni and arsenite resistance.

D. Comparison of the proteins expressed in response to Ni and As (III)

A comparison of the proteome of *Anabaena* PCC7120 responsive to Ni and As (III) identified in this study was achieved based on the gene ID. The results obtained revealed that 25 proteins were commonly differential in response to the metals studied (Table III). Most of the proteins showed a similar expression pattern, 1) ATP Synthase B subunit (atpB gene product), inorganic pyrophosphatase, 30S ribosomal protein S1, translation elongation factor EF-Ts, translation elongation factor EF-Tu, polyribonucleotide nucleotidyl transferase, ATP dependant clp protease, chaperonin GroEL, heat shock Protein class J, SOD-B, Fe-SOD, Mn containing catalase, ATP dependant clp protease, oxidoreductase, peptidyl prolyl cis trans isomerase B, peroxiredoxin, alr0803, all3324 and all4050 demonstrated up-accumulation under both stresses suggesting common induction of antioxidative defense pathways and enhanced protein synthesis and refolding. Similarly Rubisco large subunit, glutamate ammonia ligase, NADH dehydrogenase chain J, uroporphyrinogen decarboxylase, phycobilisome core component, fructose 1,6 –bisphosphatase, phosphoglycerate kinase and glyceraldehyde-3- phosphate dehydrogenase showed down-accumulation indicating inhibitory effect on photosynthetic processes and carbon metabolism.

Table III: List of the identified proteins categorized into two major groups on the basis of their performance under Ni and As(III) stresses

Proteins upregulated by Ni and As(III)	**Gene ID**
ATP Synthase *B* subunit (atpB gene product)	all5039
Inorganic pyrophosphatase	all3570
Fructose 1,6 –bisphosphatase	alr1041
Phosphoglycerate kinase	all4131
Glyceraldehyde-3- phosphate dehydrogenase	all5062
30S ribosomal protein S1	all0136
Translation elongation factor EF-Ts	all4791
Translation elongation factor EF-Tu	all4337
Polyribonucleotide nucleotidyl transferase	all4396
ATP dependant clp protease	all4358
Chaperonin GroEL	alr3662
Chaperonin GroEL	alr1896
Heat shock Protein ,classJ	alr1809
SOD-B,Fe-SOD	alr2938
Mn containing catalase	alr3090
ATP dependant clp protease	all4357
Oxidoreductase	alr5182
hypothetical	all4050
Peptidyl prolyl cis trans isomerase B	all4287
Peroxiredoxin	alr4641
Hypothetical	alr0803
Proteins downregulated by Ni and As(III)	
Rubisco large subunit	alr1524
Glutamate ammonia ligase	alr2328
Nutrient stress induced DNA binding protein	alr3808
NADH dehydrogenase chain J	all3840

Furthermore, ferridoxin NADP reductase and nutrient stress induced DNA binding protein were up-accumulated in nickel stress however showed opposite expression under As(III) stress. Ferridoxin NADP reductase is involved in photosynthesis and oxidative stress protection and possibly cells increases the abundance of this protein to control Ni stress effects. However as As(III) is more toxic to the cell and it may be more difficult for *Anabaena* to survive under As(III) stress. A hypothetical protein all4050 enhanced accumulation in response to Ni and As(III). This protein could be an interesting candidate for future functional studies to determine its relation to metal stress.

CONCLUSION

The results obtained in present study demonstrates that several proteins were affected after the addition of Ni and As(III) to *Anabaena* cultures. In response to both treatments, a common decline in proteins related to carbon metabolism and photosynthesis could be witnessed. Up-accumulation of hypothetical proteins all4050, alr0803 under both stresses calls for further investigation under both stresses. Furthermore, differences in cells to cope up with both stresses were also

observed. Present study is the first report of the global protein profile of *Anabaena* PCC7120 in response to Ni and As(III) stress. Overall, the results obtained in this study give an insight into the proteins with altered abundance in response to Ni and As(III) in *Anabaena* and can provide basic information for future studies aiming at the elucidation of Ni and As stress resistance mechanisms in heterocystous cyanobacteria.

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