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Cadmium and UV-B induced changes in proteome and some biochemical attributes of *Anabaena* sp. PCC7120

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Abstract

Impact of cadmium (Cd) and UV-B applied individually as well as in combination was investigated on the cyanobacterium *Anabaena* sp.PCC7120 using growth, pigment composition, antioxidants, peroxide production, ATP, NADPH, GSH pool, thiol content and protein profile. UV-B was found to increase peroxide production more significantly than cadmium while a significant decline in chlorophyll and phycocyanin was observed in it. Cadmium produced more pronounced effect on the energy state and total thiol content in the organism. SOD and catalase registered a maximum increase in UV-B while APX and GR were found to be highest in response to cadmium. Further, 2-DE gel of control, Cd, UV-B and Cd+UV-B revealed 453, 386, 273 and 233 spots respectively. Eleven significantly altered spots were identified using MALDI/TOF-MS. Of these FeSOD, Prx, AhpC belonged to antioxidative defence system; DnaK, GroEL, EF-Ts, 30S ribosomal protein s1, polynucleotide phosphorylase/polyadenylase to protein folding and synthesis and alr0882 to hypothetical category. An overall assessment of the two stresses using biochemical and proteome data revealed a more pronounced inhibition in UV-B than cadmium when used singly. However, its combination with cadmium produced a less deleterious effect. The biochemical and proteomic attributes suggests the antagonistic effect of these two stresses.

Introduction

Cyanobacteria are one of the most primitive prokaryotes inhabiting almost all biological niches including extreme environments (Noaman et al., 2004). Diazotrophic species like *Anabaena* fix atmospheric nitrogen and enhance crop productivity. These nitrogen fixing cyanobacteria are continuously exposed to the environmental stresses like heavy metals, salinity, UV-B radiation, heat, drought etc. Among these Cd and UV-B are the two most important stresses known to affect the cyanobacteria (Bhargava et al., 2007). *Anabaena* sp.PCC7120, used in this study is a filamentous diazotroph with a completely sequenced genome.

Cd is a non-essential metal which finds its way into the ecosystem through a variety of sources such as industrial pollution (25000 tones/year), circulation of sewage sludge and commercial phosphate fertilizers (Weisberg et al., 2003; Adams, 2004; Iribar, 2000). Cd is reported to induce morphological variation as reported in *Phormidium* (Surosz and Palinska, 2000), *Anabaena flos-aquae* (Surosz and Palinska, 2004), *Nostoc muscorum* (Bekasova et al., 1999) etc. along with the inhibition of various physiological processes such as growth, photosynthesis, respiration, nitrogen fixation and uptake of nutrients (Rai et al., 1991; Atri and Rai, 2003; Lagiriffoul et al., 1998; Siedlecka and Krupa, 1999; Sandalio et al., 2001; Ramos et al., 2002; Surosz and Palinska, 2004) which ultimately affect its survival. Though Cd is not a redox active metal, its effect on electron transport chain and photosynthetic efficiency results in an oxidative burst leading to oxidative damage (Rai et al., 1991). The toxicity of Cd is due to its high affinity with sulfhydryl groups of proteins. Low molecular weight proteins like phytochelatin, and metallothienin are reported to be involved in Cd toxicity in case of *Anabaena doliolum*, *Synechococcus* and *Scenedesmus* (Mallick et al., 1994; Turner and Robinson, 1995; Nagalakshmi and Prasad, 2001). Cadmium interferes with homoeostasis of essential metals such as Zn, Ca, Fe, Mg and Cu which are cofactor of proteins and thus affect the protein profile of the organism. While Cd induced proteomic changes have been investigated in *E. coli* (Carla et al., 2007), *Saccharomyces serevisiae* (Vido et al., 2001), *Schizosaccharomyces prombe* (Bae and Chen, 2004), *Nannochloropsis oculata* (Kim et al., 2005), *Chalmydomonas reinhardtii* (Gillet et al., 2006), and *Arabidopsis thaliana* (Roth et al., 2006), its impact has not yet been examined on the proteome of the cyanobacterium *Anabaena* sp. PCC7120.

UV-B is another potential abiotic stress negatively affecting crop productivity and living organisms. Photosynthetic organisms including cyanobacteria are highly prone to UV-B which causes oxidative damage to protein, nucleic acid and lipids (Agrawal and Rathore, 2007; Lizana et al., 2009). Since cyanobacteria are predominantly photoautotrophic organisms growing luxuriantly in tropical paddy fields, they are often exposed to high UV-B radiation (Sinha et al., 1999). UV-B not only alters the motility and photo orientation in cyanobacteria (Donker and Hader, 1991) but also affects a number of biochemical and physiological processes such as growth, survival, pigmentation and protein profile of the organism

(Aratoz and Hader, 1997). It inhibits the enzymes nitrogenase and glutamine synthetase (Kumar et al., 1996; Sinha et al., 1995). Cyanobacteria have a variety of mechanisms to cope up with UV-B radiation such as avoidance by moving into low UV-B flux, antioxidative defense enzymes, altering composition and thickness of protective sheath and synthesis of secondary metabolites like MAAs (microsporin like amino acids) and scytonemanin. Proteomic analysis of UV-B induced changes in *Synecchocystis* sp. PCC6803 suggest that significantly altered proteins belong to amino acid biosynthetic pathways, photosynthesis, respiration and energy metabolism (Gao et al., 2009).

Interactive effect of Cd and UV-B radiation is reported to inhibit growth and photosynthesis in *Anabaena doliolum* (Bhargava et al., 2007) and *Plectonema boryanum* (Prasad et al., 2005). Both Cd and UV-B have been demonstrated to stimulate the formation reactive oxygen species at various points of the photosynthetic and respiratory electron transport chain and induces lipid peroxidation (Arora et al., 2002; Ambasht and Agrawal, 2003; Zhang, 2010). The present paper is an attempt to evaluate the individual and combined affects of UV-B and Cd in the cyanobacterium *Anabaena* sp. PCC7120 (referred as *Anabaena* hereafter) using biochemical and proteomic approaches. 2DE followed by MALDI-TOF/MS analysis was used to identify the significant alterations in the protein profile of the organism.

Materials and methods

Organism and growth conditions

The cyanobacterium *Anabaena* sp. PCC 7120 was grown photoautotrophically in BG-11 medium buffered with Tris/HCl at 25 ± 2 ^{0}C under day light 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.

Experimental setup and treatment

The LC_{50} dosage for Cd and UV-B were 10µM ($CdCl_2$) and 30 min respectively for *Anabaena* as determined by the plate colony count method (Rai and Raizada, 1985). A 100 µM stock solution of $CdCl_2$ was used for Cd treatment. The UV-B treatment was given by a UV-B lamp (CAT No. 34408, Fotodyne Inc., New Berlin, WI, USA). UV-B dose of 9.6 kJm^{-2} was obtained by adjusting the distance between the UV-B source and the sample. The UV-B irradiance was measured with a Black-Ray J-221, longwave ultraviolet intensity meter (UVPInc., San Gabriel, CA, USA). All the samples (treated and control) were grown under similar conditions in triplicate to ascertain reproducibility of the results.

Growth measurement

Growth was estimated by measuring the absorbance (optical density) of the cyanobacterial culture at 750 nm in a UV–VIS spectrophotometer (Systronics, India) on every third day up to 15 days.

Pigment estimation

The control and treated *Anabaena* cells were harvested by centrifugation, dried and subjected to chlorophyll extraction for overnight in 80% acetone (spectroscopy grade). Chlorophyll and carotenoid were measured at 663 nm and 480 nm respectively in UV/Vis spectrophotometer and the concentration was determined using specific absorption coefficient (Jiang and Zhang, 2001). Phycocyanin content was estimated following the method of Seigelman and Kycia (1978). The pellet obtained was crushed in 50mM potassium phosphate buffer (pH 6.8) and absorbance was measured at 615 nm and 652 nm.

Measurement of ATP and NADPH/NADH content

The size of ATP pool was measured by the method of Larson and Olsson (1979). Control and treated cells incubated for a known period were withdrawn, centrifuged and treated with trichloroacetic acid (TCA). The sample was diluted with Tris buffer to a final TCA concentration of <0.01%. The ATP content was measured by Luciferin-Luciferase assay using LKB 1250 luminometer. The NADPH/NADH content was measured by extraction in Tris–Cl (pH 8.0) using ultrasonicator. This was subjected to centrifugation at 6500 rpm for 15 min at 4^{0}C and the supernatant was collected. The NADPH/NADH content was measured at 340 nm (Mi et al., 1995).

Measurement of lipid peroxidation (TBARS content), total peroxide, GSH and thiol

Oxidative damage of lipid was measured in terms of the total content of 2-thiobarbituric acid reactive substances (TBARS) using the method of Cakmak and Horst (1991). The total peroxide was measured according to Sagisaka (1976). Total thiol content was estimated according to the method of Ellman (1959). Total GSH content was measured according to method of Anderson (1985).

Enzymatic assays

For assay of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate

peroxidise (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) cell extract was prepared by sonication in lysis buffer under ice cold condition. The cell lysis buffer contained 1 mM EDTA and 1% (w/v) poly vinyl pyrrolidone (PVP) with the addition of 1 mM ascorbate in case of APX assay. The sonicated sample was centrifuged at 4^{o}C and the resulting supernatant was used for the assay of antioxidant enzymes. SOD activity was assayed by monitoring the inhibition in reduction of nitro blue tetrazolium (NBT) according to the method of Gianopolitis and Ries (1977). CAT activity was determined by measuring the consumption of H_2O_2 (extinction coefficient 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min (Aebi, 1984). Activity of GR was determined as per the method of Schaedle and Bassham (1977). APX activity was determined by measuring the decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) for 1 min in 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM Ascorbic acid, 0.1 mM H_2O_2, and 200 µl of enzyme extract (Nakano and Asada 1981). Total soluble protein was measured by the method of Bradford (1976).

Total protein extraction and 2DE separation

Protein extraction was performed according to a modified protocol of Wagner et al., (2002). Control and treated *Anabaena* cells harvested by centrifugation, washed with Tris buffer (pH 8.0) and suspended in 2 ml extraction buffer containing 10 mM Tris–HCl (pH 8.0), 1.5 mM $MgCl_2$ and 10 mM KCl. Cell pellet was ground in liquid nitrogen followed by centrifugation at 9200xg for 1 h. The supernatant obtained was precipitated with 10% TCA in acetone, left overnight for precipitation and centrifuged at 6000g for 15 min to recover protein pellet. This was washed 3 to 4 times with ice cold acetone to remove traces of TCA. The air dried pellet was dissolved in rehydration buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 40 mM DTT (DL-dithiothreitol), and 1.0% IPG buffer (4-7). Protein content was estimated according to Bradford using BSA as standard. Before loading for 2-DE traces of bromophenol blue was added and centrifuged at 19000g for 10 min. Sample entry was made through in-gel rehydration. A total of 300 µl of solubilization buffer containing 250 µg protein sample was incubated with the dry IPG (immobilized pH gradient) gel strips (pH 4–7 linear gradients 13cm; GE healthcare, USA) at 20^{0}C for 16 h. The first dimension separation was conducted at 20^{0}C with an Ettan IPG phor system (GE Healthcare BIO-Science, USA). Focusing was performed in 7 steps: linear 30V for 00:30 h, 150V for 2h, 300V for 00:40 h, 500V for 4h, gradient 1000V for 1h, gradient 8000V for 2h and finally 8000V for 13h. Focused IPG strips were then equilibrated by first incubating them in an equilibration solution (6M urea, 30% v/v glycerol, 2% w/v SDS, 50 mM Tris-HCl, pH 8.8) having 1% w/v DTT and a trace amount of bromophenol blue) for 15 min, followed by 15 min incubation with 2.5% w/v iodoacetamide. The strip was placed on the top of 12% SDS-polyacrylamide gels and sealed with 1% agarose. Electrophoresis was carried out at 10 mA for 30 min, then 25 mA for 5h using a Hoefer SE 600 apparatus (Amersham Biosciences, USA). The gel was stained with coomassie brilliant blue R-250 (CBB).

Image analysis

The gels were scanned and protein spots were analyzed using PDQuest software version 7.1 (Bio-Rad). Spot intensities were calculated and normalized by determining the relative intensity of each spot (% volume) by dividing the intensity of each spot to the sum of the intensities of all spots on the corresponding gel. 25 spots showing significant and reproducible changes were selected, excised using a sharp and sterile razor blade and subjected to MALDI-TOF/LC-MS analysis followed by homology search using MASCOT. The MALDI-TOF identification results are given as supplementary data. The Molecular weight of the proteins on gels was determined based on standard protein markers (Bangalore Genei) and pI was determined by the migration of protein spots along the 13 cm IPG strips (4–7 linear). The protein databases employed were, NCBInr (The National Center for Biotechnology Information non-redundant) and Swiss-Prot. In brief, 1 µl of trypsinized peptide samples were mixed with matrix, namely α-cyano-4-hydroxy cinnamic acid (CHCA). Following drying, the peptides were spotted on ground steel plate and subjected to Bruker Ultra flex MALDI-TOF/TOF and 2D Nano LC-ESI-Trap (Agilent) for mass spectrometric identification. Data acquisition and analysis were performed using flex control and flex analysis/biotools version 2.2 software, respectively (Bruker Daltonics). All spectra were smoothened, and all peptide mass fingerprint spectra were internally calibrated. Then, the measured tryptic peptide masses were transferred through MS BioTool program as inputs to search against the taxonomy of other bacteria in the databases using MASCOT 2.0 software (Matrix Science). The peptide mass fingerprinting (PMF) and MS/MS ion search were searched with the following MASCOT settings: taxonomy as bacteria; peptide mass tolerance of ±100 ppm for peptide mass fingerprinting and ±1.2Da for MS/MS, monoisotopic

mass, alkylation of cysteine by carbamidomethylation as a fixed modification, and oxidation of methionine as a variable modification.

Results

Growth measurement and pigment estimation

Figure 1 shows the growth behaviour of control and treated *Anabaena* at individual and combined doses of Cd and UV-B. A decline of 29.8%, 49.0 % and 39.0% in specific growth rate was observed in Cd, UV-B and Cd+UV-B respectively with respect to control. Figure 2(A) demonstrates changes in pigment content in control and treated samples. A decline of 32.7%, 42.9% and 35.92% for Cd, UV-B and Cd+UV-B respectively was observed in chlorophyll as compared to control. Similarly, a decline of 22.52%, 66.85% and 51.44% was observed for phycocyanin. However, carotenoid content was decreased by 35.17%, and 30.29% in Cd and Cd+UV-B respectively and an increase of 16.41% was recorded in UV-B.

Total peroxide, TBARS (lipid peroxidation), GSH, and thiol content

Figure 2(B) compiles data on total peroxide and lipid peroxidation (TBARS content) in control and treated *Anabaena* cells. An increase of 62.45%, 79.40% and 45.60 % in total peroxide and 40.65%, 63.50% and 14.5% in TBARS content was recorded for Cd, UV-B, Cd+UV-B respectively. Similarly, GSH was observed to increase by 65.33%, 11.12% and 33.25% and thiol by 34.35%, 12.64%and 11.45 % under Cd, UV-B and Cd+UV-B treatments respectively as compared to control (Fig. 2C).

Enzymatic assays (SOD, CAT, APX, and GR)

All tested enzymes showed a significantly enhanced activity in Cd and UV-B treatments as compared to control. SOD showed an increase of 32.17%, 45.78% and 9.15% in Cd, UV-B and Cd+ UV-B treatments with respect to control. Maximum CAT activity of 38.29% was recorded in UV-B followed by 21.57% and 8.78% in Cd and Cd+ UV-B. A similar trend in activity was observed for both APX and GR. A 70.05%, 40.20%, and 18.48% increase in APX and 31.08%, 11.36% and 19.35% increase in GR was observed in Cd, UV-B and Cd+UV-B respectively (Fig.2D).

NADPH and ATP content

NADPH and ATP levels of control, Cd, UV-B and Cd+UV-B treated *Anabaena* are presented in Figure 2E. 5.1%, 40%, and 12.67% increase in NADPH was observed in UV-B, Cd and Cd+UV-B. Similarly, the ATP content showed decrease of 51.39%, 33.65% and 26.80%% in Cd, UV-B and Cd+UV-B respectively as compared to control.

Protein profile in response to Cd and UV-B stresses

A total of 453, 386, 273 and 233 spots were analysed by PDQuest software in control, Cd, UV-B and Cd+UV-B respectively in 2-DE gels after 24 h treatment. Out of 453 spots in the control gel, 127, 130 and 139 spots were found to match with those in Cd, UV-B and Cd+UV-B respectively. 10 differentially expressed proteins were subjected to MALDI-TOF/MS analysis followed by MASCOT search (Table 1). The identified proteins were DnaK, GroEL, transketolase (TK), fructose 1,6 bisphosphate aldolase (FBPaldolase), EF-Ts, peroxiredoxin(Prx), AhpC/TSA protein, alr0882, polynucleotide phosphorylase/polyadenylase (PNPP/PA), Fe-SOD and 30s ribosomal protein s1 (30 s RP s1).

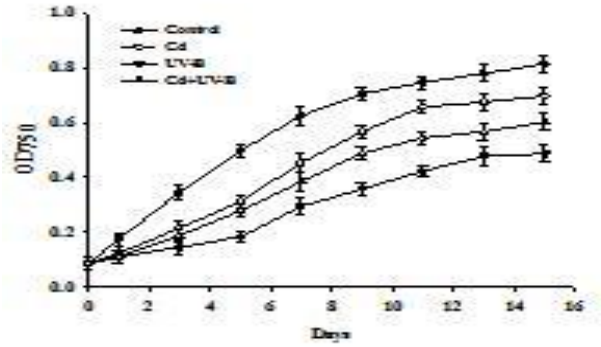

Fig. 1 Growth behaviour of *Anabaena* PCC 7120 subjected to (i) Control (ii) Cd (iii) UV-B (iv) Cd +UV-B after 24 h. Value is given as mean±SE

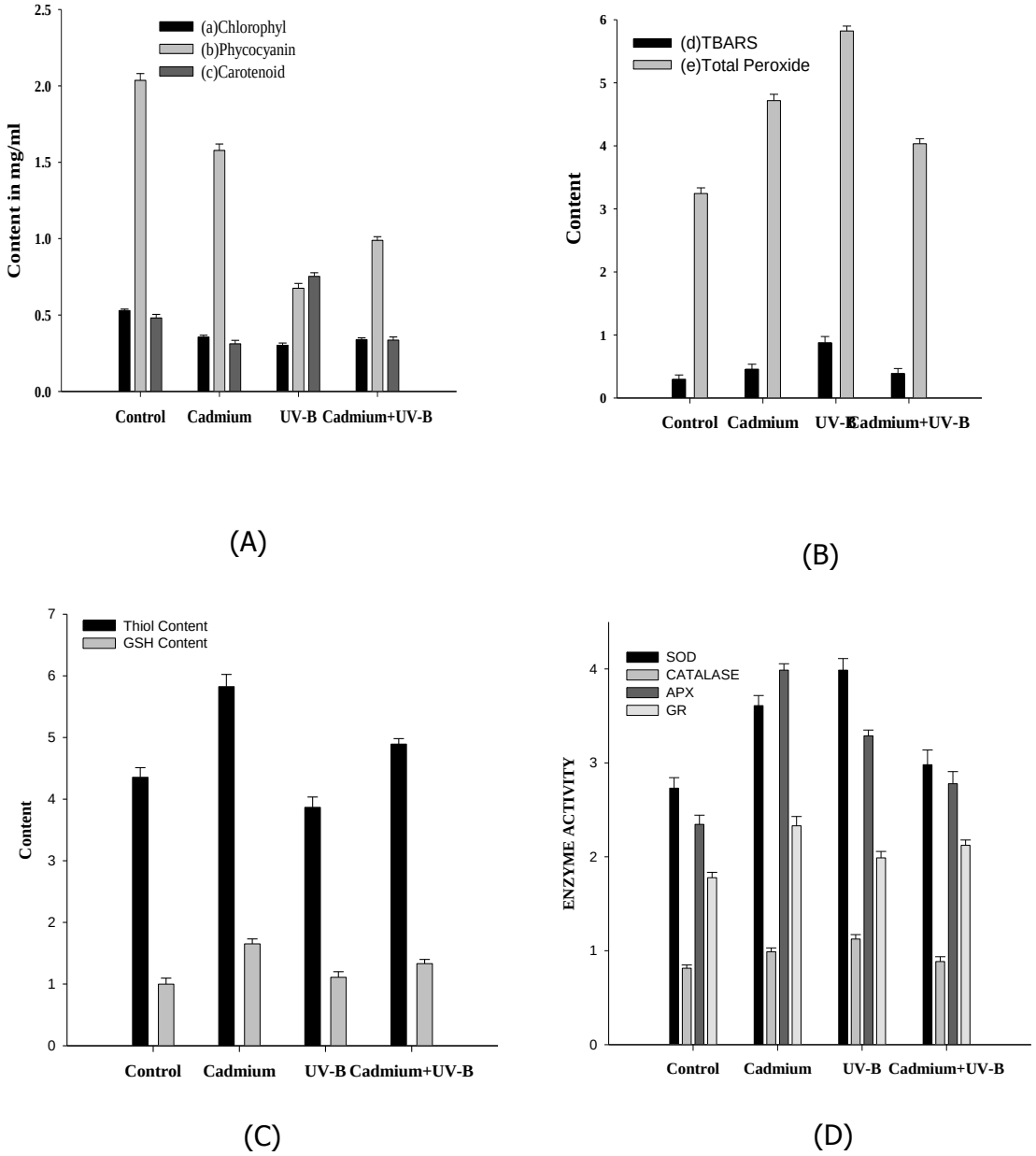

Fig. 2 (A) Chlorophyll, phycocyanin and carotenoid content (in mg/ml) (B) TBARS and total peroxide (in mM/mg protein) (C) Thiol and GSH contents (in nM/mg protein) (D) SOD (U enzyme/mg protein), CAT (µM/min/mg protein), APX (nM/min/mg protein) and GR (mM/min/mg protein).

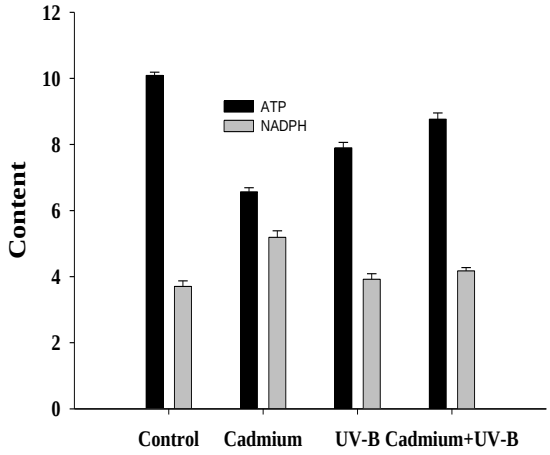

Fig. 2(E) ATP (µg/mg of protein) and NADPH (mM/mg of protein)

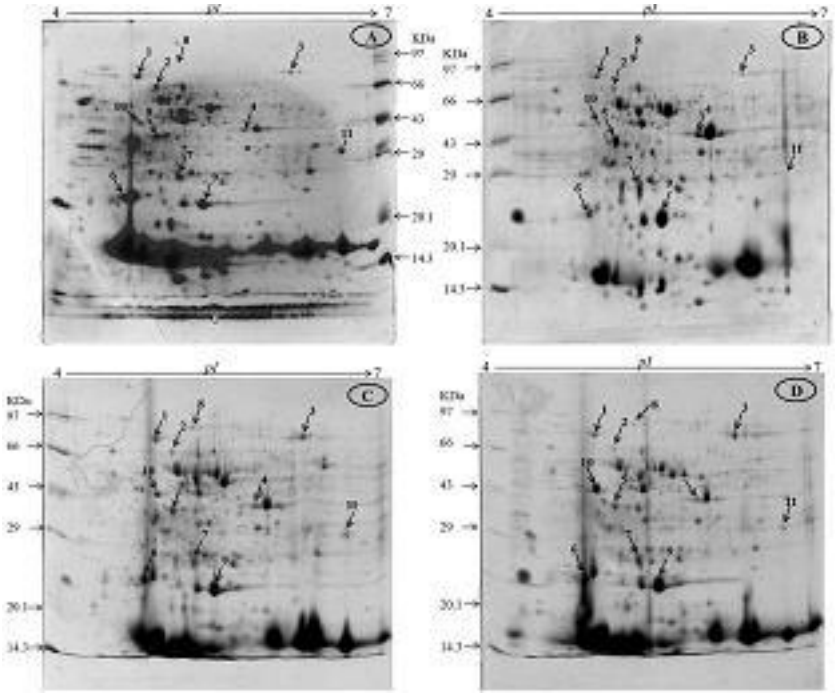

Fig. 3 The 2-DE images of total cytosolic protein extract from *Anabaena* (A) control (B) 10µM $CdCl_2$ (C) 30 min of UV-B exposure and (D) $CdCl_2$ + UV-B. All gels were run in triplicate. Proteins were extracted and separated by 2-DE, and visualized by CBB staining. Untreated *Anabaena* was used as control. The protein (400µg) was applied to pH 4–7 IPG dry strips with 12% linear vertical SDS PAGE as the second dimension. 10 proteins were analyzed by MALDI-TOF / MS. Details are given in Table 1.

Table 1. Cd and UV-B responsive differentially expressed proteins in *Anabaena* sp. PCC7120 identified by MALDI-TOF/ LC-MS

Spot No.	Protein name (showing homology with)	*M*r (kDa)/ p*I* Theor.	*M*r (kDa)/ p*I* Exper.	Accession no.	No. of peptides matched	Mowse score
1.	DnaK (*Nostoc* sp.strain PCC7120)	8.04/4.7	70.0/4.8	gi|17229234	29	196
2.	GroEL (*Nostoc* sp.strain PCC7120)	8.93/4.8	64.0/5.0	gi|17229388	19	127
3.	Transketolase (*Nostoc* sp.strain PCC7120)	2.31/5.9	72.0/6.0	gi|17230836	27	167
4.	Fructose 1,6 bis phosphate aldolase (*Nostoc* sp.strain PCC7120)	8.76/5.4	39.0/5.7	gi|17232055	14	111
5.	EF-Ts (*Nostoc* sp.strain PCC7120)	34.43/-	33.2/5.1	gi|17232283	9	114
6.	Peroxiredoxin (*Nostoc* sp.strain PCC7120)	2.73/4.7	21.5/4.7	gi|17232133	10	95
7.	AhpC TSA family protein (*Nostoc* sp.strain PCC7120)	23.74/-	25/5.6	gi|17231896	7	267
8.	Polynucleotide phosphorylase/polyadenylase (*Nostoc* sp.strain PCC7120)	77.86/4.9	80.0/5.2	gi|17231888	17	138
9.	Fe-SOD (*Nostoc* sp.strain PCC7120)	22.37/5.2	22/5.6	gi|17230430	7	285
10.	30 S ribosomal protein S1 (*Nostoc* sp.strain PCC7120)	38.34/4.5	1.0/4.6	gi|17227632	13	88
11.	alr0882 (*Nostoc* sp.strain PCC7120)	30.88/7.7	27.5/7.6	gi|17228377	10	100

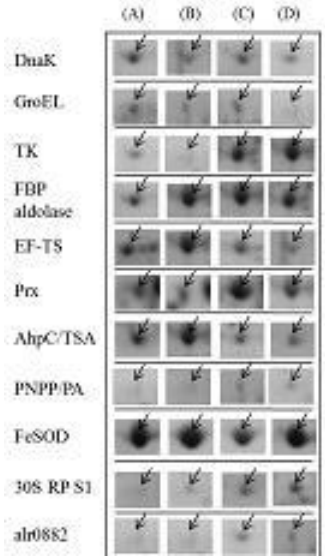

Fig. 4 Changes in abundance of selected proteins of (A) Control (B) Cd (C) UV-B (D) Cd +UV-B. Details of the proteins are given in Table1.

Discussion

The results of the biochemical and proteomic data suggest a more pronounced damaging effect of UV-B in comparison to Cd (Hernandez and Almansa, 2002). In the present study Cd and UV-B were applied individually as well as in combination on *Anabaena*. Interestingly, most of the biochemical parameters and also the growth behaviour indicated that Cd and UV-B when applied in combination were less damaging than individual Cd and UV-B treatments (Fig. 1).

A more pronounced decrease in chlorophyll was observed in UV-B than Cd (Fig. 2A). This finds support from the study of Bhargava et al., (2007). Chlorophyll degradation in Cd may be due to inhibition of chlorophyll biosynthesis pathway (Larsson et al., 1998). In comparison to Cd and Cd+UV-B, carotenoid content was found to be highest in UV-B (Fig. 2A). Carotenoids act as filter for UV-B radiation (Jürgens and Weckesser, 1985). They are effective singlet oxygen quenchers having ability to eliminate activated oxygen radicals hence provide protection against photooxidative damage therefore their induction in UV-B stress is quite logical. The enhanced carotenoid in UV-B could further be linked to the induction of 3-hydroxy-3 methyl glutaryl reductase RNA for the synthesis of carotenoids (Campos et al. 1991). Phycocyanin content was maximally inhibited in UV-B alone as it is extremely sensitive to UV radiation (Quesada et al., 1995) in comparison to Cd and Cd + UV-B.

In this study, the oxidative stress indicators like TBARS and peroxide content were significantly evident in case of UV-B suggesting that UV-B alone produced more redox imbalance in comparison to Cd and Cd+UV-B treatments. In UV-B stress, production of toxic oxygen radicals may oxidize the fatty acids leading to lipid peroxidation and disturb the ratio of saturated/unsaturated fatty acid and hence membrane fluidity (Kramer et al.1991). In contrast to this Cd induced lipid peroxidation is an indirect consequence caused by hydrogen peroxides accumulated due to inhibition of various metabolic pathways (Prasad and Zeeshaan, 2005). It is worthwhile to mention that increased generation of reactive oxygen species was associated with enhanced activities of ROS scavenging enzymes like SOD, CAT, APX and GR.

A significant increase in activity of SOD in all the treatments, maximum in UV-B, indicate scavenging of oxygen free radicals and dismutation of free hydroxyl radicals by the formation of hydrogen peroxide (Mackerness et al., 2001). Further, to detoxify the hydrogen peroxide, enhanced activities of catalase and APX in both the stresses appear reasonable. CAT activity was found to be significantly higher in case of UV-B alone suggesting removal of H_2O_2 and toxic peroxides. Glutathione reductase (GR), which catalyses the NADPH-dependent reduction of oxidized glutathione, showed significant increase in activity like other enzymes and can be directly corroborated with APX involved in the Halliwel-Asada pathway. GR and APX activities were maximally observed in case of Cd. All antioxidant enzymes such as - SOD, CAT, APX and GR showed increase reflecting their key role in cell survival and tolerance against Cd and UV-B stresses (Halliwell and Gutteridge, 2006).

Thiols and GSH are known to play a role in maintenance of redox status as well as in the detoxification of metals (Hartley-Whitaker et al., 2001). GSH acts as an antioxidant, quenching the ROS generated in response to various abiotic stresses (Gill and Tuteja 2010). The high GSH content in Cd and Cd+UV-B in contrast to UV-B alone is probably due to transcriptional activation of *gsh*1 and *gsh*2 genes coding for GSH (Xiang and Oliver 1998), which are required for phytochelatin synthesis and Cd sequestration. In addition to this, due to the presence of thiol groups, GSH can also act as a defense against oxidative stress. The sensitivity of GSH to UV-B may be because it absorbs in the range of 200-300 nm (Tyagi *et al.* 2003) leading to its oxidation. Similarly high thiol content in Cd and Cd+UV-B might be attributed to its role as an antioxidant and in protecting membranes against free radical damage (Rennenberg, 1994; Nagalaxmi and Prasad, 2001).

Further for better understanding of Cd and UV-B mediated cellular response of *Anabaena*, the protein profile was done by 2-DE coupled with MALDI/TOF-MS. Out of eleven significantly altered protein spots, FeSOD, Prx and AhpC/TSA belonged to the antioxidative class of proteins. Upregulation of FeSOD and Prx is in accordance with the reports of Bhargava et al., (2007) and Mishra et al., (2009) respectively. The upregulation of FeSOD on 2-DE gel attests the enhanced SOD activity which was biochemically measured in different treatments. Prx and AhpC/TSA are the important proteins required for the scavenging of ROS and RSS produced under stress and their over-expression is essential for combating the stress induced oxidative damage. Similarly AhpC was upregulated in Cd and Cd+UV-B but downregulated in UV-B as reported in arsenic stress (Pandey et al., 2011).

Two chaperons-DnaK and GroEL, identified in the protein profile function for denovo folding/ refolding of stress induced denatured/ aggregated proteins

(Hoffmann et al., 2010). Down regulation of both DnaK and GroEL in all treatments after 24h might be correlated with the decrease in ATP content as the activity of molecular chaperones requires cycles of ATP regulated binding and release (Hoffmann et al., 2010). An increase in the level of 30s ribosomal protein s1, the protein involved in ribosomal assembly was upregulated in Cd, UV-B and Cd+UV-B treatments with respect to control suggesting that Cd and UV-B affect transcription and translation process of the test organism. Up regulation of FBP aldolase in Cd and UV-B treatments with respect to the control indicates enhanced respiration under both the stresses. While UV-B upregulated the transketolase (TK), downregulation was observed in Cd as compared to control. TK is an amphibolic enzyme required in pentose phosphate pathway (PPP) and Calvin-Bensen cycle. Upregulation of TK in UV-B along with increase in NADPH level might be due to operation of pentose phosphate pathway in the cell (Gao et al., 2009). Hypothetical protein alr0882, upregulated in UV-B and Cd+UV-B finds support from the study in *Anabaena doliolum* against UV-B treatment (Mishra et al., 2008). Blast P search for alr0882, indicated the possibility of its being a universal stress protein (USP) as it shares 96.8% homology to uspA of *Anabaena variabilis* ATCC 29413 and harbors two uspA domains within it. Thus, an overall assessment of biochemical and proteomic data suggest that negative effects of Cd and UV-B produced individually are appreciably mitigated when applied in combination. This may therefore be not as damaging to cyanobacteria and crops as one might visualize.

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