Int J Pharm Bio Sci 2012 July; 3(3): (B) 956 - 966

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANTIOXIDATIVE POTENTIAL OF CATALASE IN BLOOM FORMING CYANOBACTERIA- *ANABAENA VARIABILIS* AND *SYNECHOCOCCUS ELONGATES*

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ABSTRACT

Cyanobacteria are fascinating and versatile group of bacteria which are of immense biological importance. They are unique photosynthetic prokaryotes with an oxygenic photosynthesis process. They possess an array of protective mechanism comprising of antioxidants to eliminate the damages caused by the reactive oxygen species. Blooms caused by cyanobacteria are ubiquitous in temple ponds, lakes and other water reservoirs. In this study, the antioxidative potential of catalase in bloom forming cyanobacteria *Synechococcus elongatus* and *Anabaena variabilis* was determined. The activity of catalase by spectrophotometric analysis, using hydrogen peroxide as the substrate showed activity 35.45±4.45mM/min/µg protein in *Synechococcus elongatus* and 13.68±1.09 mM/min/µg protein in *Anabaena variabilis.* The presence of catalase was also confirmed by native–PAGE, discrete band was visualized after activity staining. Antioxidants have the potential to serve significant role in processing and storage of food and other medicinally important preparations such as dry vaccines. Research on occurrence of antioxidative enzymes and elucidating the mechanisms behind the stability of these enzymes in various systems will open new horizons.

KEYWORDS: **Cyanobacteria, Antioxidants, Catalase, Blooms, Reactive oxygen species.**

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INTRODUCTION

Cyanobacteria are unique photosynthetic bacteria that occur throughout the world (Al Kahtani *et al*., 2008). They are supposed to have evolved around 3.5 billion years ago are the first oxygen evolving organisms to release oxygen leading to the development of aerobic metabolism and the subsequent rise of higher plants and animal forms (Minu *et al.* 2011). Photosynthesis and respiration in aerobic organisms generate free radicals and antioxidative enzymes play a pioneer role in scavenging free radicals. Cyanobacteria possess enzymatic and non- enzymatic antioxidants in which enzymatic includes catalase, superoxide dismutase, glutathione, ascorbate and non-enzymatic includes pigments like phycobilliproteins and chlorophyll. These antioxidants act against the reactive oxygen species (ROS) that are commonly generated during growth and development as well as during normal cell metabolism related with oxidation and reduction processes. ROS are formed as a result of thylakoids, mitochondria and plasma membrane linked transport and subsequent leaking of electron to the molecular oxygen in the cell (Minu *et al.* 2011). In metabolic systems, when oxygen comes in contact, it can be transformed into more reactive and toxic form of superoxide ion, hydrogen peroxide, hydroxyl radical and singlet oxygen. The further production of reactive oxygen species such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl (OH^-), and per hydroxyl radicals (O_2H) is stimulated by formation of singlet oxygen (O_2).

Catalase (EC 1.11.1.6) is one of the most potent catalysts known. Catalase catalyses conversion of hydrogen peroxide, to water and molecular oxygen. Catalase performs its rapid destruction of hydrogen peroxide in two steps. Catalase also uses hydrogen peroxide to oxidize toxins such as phenols, formic acid, formaldehyde and alcohols (Scandalios *et al*., 1997; Chaudiere and Ferrari-Iliou, 1999; Karra-Chaabouni *et al*., 2003).

MATERIALS AND METHODS

Cultures

Cyanobacterial strains *Synechococcus elongatus* and *Anabaena variabilis* were isolated from natural blooms of northern India. The strains were purified in the laboratory.

Growth conditions

Synechococcus elongatus was cultured in BG-11 media (Stanier, 1971) having sodium nitrate where as *Anabaena variabilis* was cultured in BG -11 media but without sodium nitrate. The cultures were incubated in regular interval of 12 hour light and dark period.

Growth analysis

The growth of *Synechococcus elongatus* and *Anabaena variabilis* was measured spectrophotometrically at 660 nm**.** Absorbance was taken at the regular interval of five days till day 25.

Pigment analysis

Estimation of Chlorophyll a

Cyanobacterial strains were harvested by centrifugation and then 2 ml of 90% acetone was added to each of the harvested strain and then these were vigorously shaken. The centrifuged tubes wrapped in foil, were kept in refrigerator for 20-24 hours for complete extraction and later centrifuged at 3000-5000 rpm. Absorbance was taken at A (665), (645) and at (630) nm.

Phycobiliproteins

The harvested strains were grinded in pestle and mortar in the presence of phosphate buffer with sand as an abrasive for rupturing cells. After repeated freezing and thawing, the cells were centrifuged. Absorbance was taken at 562, 615 and 652nm.

Protein estimation

Protein estimation was done by using Bradford method (1976).

Enzyme Assay

Cyanobacterial cells were harvested by centrifugation (5000g for 30 min). Harvested cells were grinded in pestle and mortar in 1ml of 0.1 M phosphate buffer (pH 7) and vortexed for 15 minutes with frequent placing on ice. Centrifugation was done at 20,000g for 20 min at 4° C. A clear supernatant was obtained as crude extract. Enzyme activity was estimated by recording absorbance at 240 nm. The reaction mixture contained 3ml of phosphate buffer, 3 ml hydrogen peroxide and enzyme extract.
Native Polyacrylamide gel electrophoresis (PAGE) was performed in 10% separating gel and 5% stacking gel according to Laemmli (1970). Activity staining was done by was incubating gel in 0.003% hydrogen peroxide for 10 min and developed in a 1%(w/v) ferric chloride and 1% potassium ferrocyanide (w/v) solution for 10 min (Woodbury *et al.* 1971).

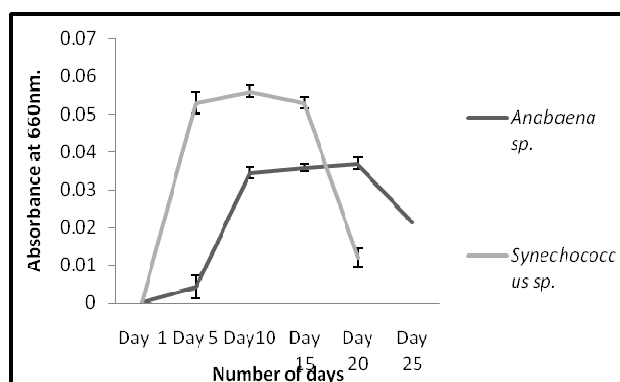
RESULTS AND DISCUSSION

Growth analysis

Cyanobacterial growth curve shows all the four phases i.e. lag phase, log phase, stationary phase and death phase. In case of *Synechococcus*, on the first day, the growth was very less but with the passage of time i.e. at day 5, growth started, which showed that the cells were now slowly adapting themselves to the changed environment. From day 5 to day 15 stationary phase was observed. But after day 15 there was the beginning of lag phase, i.e. now the cells started dying due to the lack of nutrients, oxygen deficiency, etc. In case of *Anabaena*, results were different to some extent; log phase was observed at day 10. After day 10, stationary phase was observed till 25th day and after day 25 death phase started. Growth was steady in *Anabaena* as compared to *Synechococcus*. It was reported that there was a delay phase in the *N. ellipsosporum* growth curve from day 1 to day 3, a logarithmic phase from day 3 to day 11, and a stationary phase from day 11 until day 19. The growth curve of *N. muscorum* was different and showed its delay phase from day 1 to day 5, logarithmic phase from day 5 to 13, and stationary phase from day 13 to day 19 (Moghadam *et al*., 2008). On the other hand, Lohscheider *et al.* (2011) reported that *Synechococcus* strains PE1-7 and PC1-1 had the slowest growth rate leaving the lag phase after approximately 10 to 15 days after dilution, while strains PE2-7, PE3-7, PC2-1, and PC3-05 were slightly faster and entered the growth phase after 7 to 10 days. Strain PC4-0.5 started growth already after 3 to 5 days. The doubling time during exponential growth of the cultures was between 6 (strain PC4-0.5) and 16 (strain PE3-7) days, as compared to the cyanobacterial model strain *Synechocystis* sp. PCC6803 with the culture doubling time of 6 days.

Table no. 1
Growth analysis of Synechococcus elongatus and Anabaena variabilis

Species	Day 1	Day 5	Day10	Day 15	Day 20	Day 25
Anabaena sp.	0.001±0.00	0.0043±0.003	0.0346±0.0015	0.036±0.0015	0.037±0.001	0.021
Synechococcus sp.	0.001±0.00	0.053±0.003	0.056±0.0015	0.053±0.0015	0.012±0.0026	



Graph no.1
Growth analysis of Synechococcus elongates and Anabaena variabilis

Pigments analysis
Experimental results reveal that *Synechococcus elongatus* contained higher amount of chlorophyll a than the *Anabaena variabilis*. The concentration of chlorophyll a in *Anabaena* was found to be 0.00016 mg/l and 0.00070 mg/ l in *Synechococcus* (Table-2 and Graph no.2*)*. As reported by John *et al.,* (1998), Chlorophyll *a* varied from about 20 to 280 µg·L$^{-1.}$. However, there is a great deal of confusion about which method should be used in limnological investigations, and this confusion has resulted in a number of different methods being used by various volunteer programs. Homogenization by grinding of the filter enhances the rupture of the algal cells and increases extraction efficiency of the solvent. Homogenization is an absolute necessity with an acetone solvent, but some have found that other extractants such as ethanol or methanol apparently do not need grinding to extract all the chlorophyll (Sartory and Grobbelaar, 1984). Others, however, have found that even methanol extractants do not extract as well without grinding. These other solvents are more efficient than acetone at extracting pigments from some green and blue-green algal cells. Methanol, however, is more toxic. Membrane filters can be ground but they lack the abrasiveness to produce a good extraction, and their extraction efficiencies are lowered. Spectrophotometric analysis of chlorophyll pigments were developed in the 1930's and 1940's (Weber *et al.*, 1986). Richards and Thompson (1952) introduced a trichromatic technique that was supposed to measure chlorophylls *a, b,* and *c*. Trichromatic equations attempted to remove interferences of the other chlorophylls at the maximum absorption wavelength for each chlorophyll. Since Richards and Thompson, a number of modifications have been made to these equations which purportedly produce better estimates of the chlorophylls (Parsons and Strickland, 1963;Jeffrey and Humphrey, 1975, UNESCO 1966).

Table no. 2

Pigments (mg/ml)	*Anabaena variabilis*	*Synechococcus elongatus*
Chlorophyll a	0.00016±0.00005	0.00070±0.00002
Phycocyanin	0.011±0.0036	0.010±0.004
Phycoerythrin	0.006±0.0005	0.025±0.00059
Allophycocyanin	0.023±0.004	0.021±0.0045

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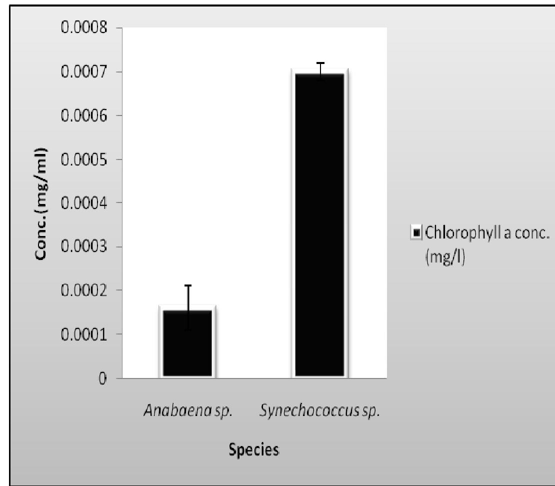
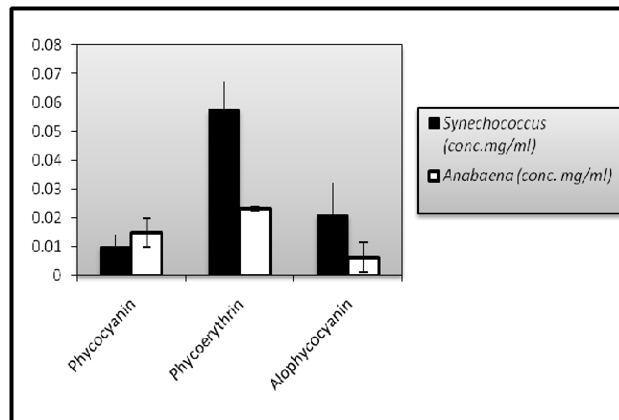


Graph no.2
Chlorophyll a analysis



Graph no. 3
Phycobiliproteins analysis

Table no.3
Protein concentration

Species	Concentration(mg/ml)
Anabaena variabilis	0.447± 0.055
Synechococcus elongatus	1.216±0.017

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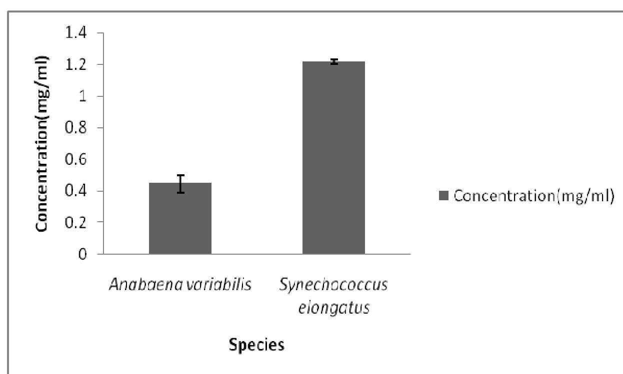

Graph no. 4
Protein analysis

The concentration of different phycobiliproteins present in the both *Synechococcus elongatus* and *Anabaena variabilis* cyanobacterial strains were estimated. Results showed that the concentration of Phycocyanin was found to be lower in *Anabaena variabilis* than in *Synechococcus elongates.* On the other hand, the concentration of Allophycocyanin was higher in *Synechococcus sp*. than in the *Anabaena* sp. The concentration of Phycoerythrin both the strains shows that *Anabaena variabilis* contains less amount of phycoerythrin than the *Synechococcus elongatus* (Table No.2 and Graph no.3). Thus, from this study it was concluded that fresh water algal blooms contain an adequate amount of pigments. These pigments can prove useful to us. We should aim to develop the methods by which we can increase the concentration of these pigments in the cyanobacterial strains for their increased utilization for various purposes.

Cyanobacteria contain abundant phycobiliproteins. Major producers of phycobiliproteins are the cyanobacterium *Arthrospira, Spirulina* and the *Rhodophyte porphyridium* (Bermejo *et al,* 2001). 6% to 46% of C-PC has been observed in *Spirulina fusiforms* (Minkova *et al.,* 2003) and 26% in *Spirulina platensis* (Zhang & Chen, 1999). *Gleotrichia natans*, a nitrogen fixing cyanobacterium as a possible source of phycobiliproteins was assessed (Boussiba, 1999). In *Nostoc* the phycobiliproteins contents reached 14% of total biomass. In a screening study by Moreno *et al*., (1995), out of 10 strains of filamentous heterocystis nitrogen fixing cyanobacteria C-PC and APC were found at 17% and 11% of dry weight in some strains of *Anabaena* and *Nostoc*. PC content in *Anabaena* species (Rodriguez *et al*., 1989), *Nostoc* sp. (Silva *et al*., 1989), *Phormidium valderianum* (Sekar & Subramanium, 1998) has been observed at 8.3%, 20% and 20% of dry weight respectively. These pigments can be employed in various purposes like markers and in cosmetics and can prove to be very useful. Many cyanobacteria have been explored for their suitability for commercial potential. *Spirulina* sp. has been used successfully to produce high concentrations of valuable compounds such as lipids, proteins and pigments (Abd El-Baky *et al.,* 2002).

Enzyme assay

To detect the presence and the activity of catalase in cyanobacterium strains Native – PAGE and spectrophotometric analysis was done. The study showed that there was remarkable presence of catalase in *Synechococcus* and *Anabaena sp*. This can be acknowledged by the Native- PAGE results (Figure no.1). Native- PAGE of purified proteins gave two bright colour discrete bands having greenish background. One dark band showed the presence of catalase in *Synechococcus elongatus* and light band of catalase was seen in case of *Anabaena variabilis*. With these bands it was concluded

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that the concentration of catalase in *Anabaena variabilis* was less as compared to *Synechococcus elongates.* Antioxidant concentration in *Anabaena variabilis* showed increase when treated with metals (Padmapriya *et al.,* 2010). Tripathi and Srivastava (2001) have reported catalase in a desiccation-tolerant cyanobacterium *Lyngbya arboricola* under dry state. The presence of two bands of catalase was detected by native PAGE in *Tolypothrix* (Rajendran *et al.,* 2007). Obinger *et. al* (1998) have demonstrated one isoform of catalase in *A. nidulans* on native polyacrylamide gel, whereas there were three isoforms of catalase recorded in *L. arboricola.* Presence of three isoforms of catalase enzyme is more common in higher plants (Rao *et al.,* 1996; Chen and Asada, 1989). This may be due to availability of more oxidative atmosphere at its subaerial habitats and also to high activity of oxygenic photosynthesis (Tripathi and Talpasayi, 1982). The level of oxygen in the cavity of *Azolla* (Canini, 1991) and bubbling cultures of *A. cylindrica* with O_2-rich air (Tel-Or, 1986) have been suggested for higher levels of AOS enzymes in these cyanobacteria. Crude extracts of green growing cyanobacterial samples collected from their natural habitats when subjected to native PAGE and stained for catalase activity showed the presence of single isoform each in *Scytonema sp., Oscillatoria sp* and *Nostoc cycadae,* while *Aulosira sp*. and *Microcystis* sp. showed presence of three isoforms of catalase (Minu *et al*. 2011). Whether the peroxidase reaction has a physiological role in destroying hydrogen peroxide would depend on the availability of suitable hydrogen donors (Hochman *et al.,* 1991). *Synechococcus* PCC 7942 catalase-peroxidase showed peroxidase activity with o-dianisidine and pyrogallol of 9.5 and 162.2 units/mg respectively (Mutsuda *et al.,* 1996).

(a) (b)

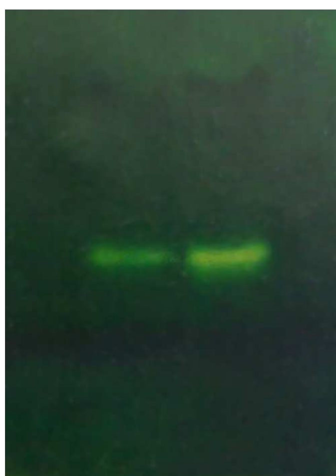


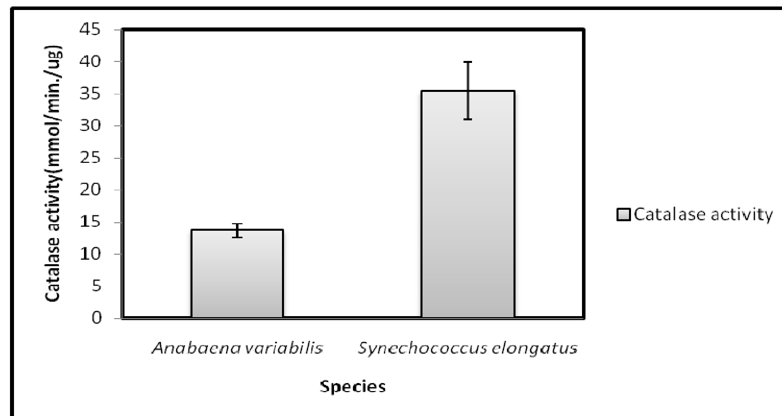
Fig no 1

Native PAGE of extract stained for catalase activity (a) Anabaena variabilis (b) Synechococcus elongates

Table no. 4

Catalase activity analysis

Species	Catalase activity (mmol/min/ug)
Anabaena variabilis	13.68±1.09
Synechococcus elongatus	35.45±4.45



Graph no 5

CONCLUSION

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Antioxidant vitamins, glutathione reductase, catalase, superoxide dismutase, 'scavenge' O_2 free radicals which otherwise cause many problems like cancer, induced DNA damage, and cardiovascular disease (Ames *et al.,* 1993). The potential therapeutic application is overwhelming in the areas of immunomodulation, anti-cancer, anti-viral, and cholesterol- reduction effects. Increased cost of health care has become a driving force in the shift towards interest in wellness, selfcare, and alternative medicine, and a greater recognition between diet and health care. Antioxidants foods rich diet decreases the risk of cardiovascular disease mortality.

β carotene has been reported to be medicinally significant being antimutagenic and protective against breast cancer. Porphyran and shinorine traced from cyanobacteria have found to play role in delaying aging process (Minu *et al.*, 2011). Phycoerythrin has been crucial in amelioration of diabetic complications (Yabuta *et al,* 2010). Antioxidants serve to provide structural and functional stability to the cells at extreme conditions. Exploration of cyanobacterial strains having antioxidative potential and their applications will prove very useful in a many avenues.

REFERENCES

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1. A. Ahmadi Moghadam and B. Nowruzi. A new report of n fixation by two species of cyanobacteria. Iranian *Journal of Science & Technology*, Transaction A, Vol. 32, No. A2 (2008)
2. Abd El-Baky, H.H., A. Moawd, A.N. El-Behairy and G.S. El-Baroty, Chemoprevention of benzo pyrene-induced carcinogen and lipid peroxidation in mice by lipophilic algae extracts (phycotene). *J. Med. Sci.,* 2: 185–93, (2002)
3. Al-Kahtani, M.A.and A.A. Fathi, Physiological studies on tilpa fish (*Oreochromis niloticus*) as influenced by the cyanobacterial toxins microcystin. *J. Boil. Sci*., 8: 1226 – 1230, (2008)
4. Ames, B. N., Shigenaga, M. K. and Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging.

Proc. Natl. Acad. Sci. U.S.A. 90, 7915-7922, (1993)

5. Bermejo Roma´n, J.M. Alva´rez-Pez, F.G. Acie´n Ferna´ndez,E. Molina Grima. Recovery of B-Phycoerythrin from the microalga *Porphyridium cruentum*. *J. Biotechnol*, 93: 73-85 (2001)
6. Boussiba, S., Bing, W., Zarka, A., Yuan, J.P. and Chen, F. Changes in pigment profiles of *Haematococcus pluvialis* during exposure to environmental stresses. *Biotechnol. Lett.* 21(7): 601-604, (1999)
7. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 72: 248-254, (1976)
8. Canini, A., Galiazzo, F., Rotilio, G. and Caiola Grilli. Superoxide dismutase in the symbiont *Anabaena azollae* strasb. *Plant Physiol*, 97, 34–40, (1991)
9. Chaudiere J, Ferrari-Lliou R Intracellular antioxidants: From chemical to biochemical mechanisms. *Food Chem. Toxicol*. 37: 949-962, (1999)
10. Chen, G. X. and Asada, K. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant Cell Physiol*., 30, 987–998, (1989)
11. Hochman, A. and Goldberg, I. Purification and characterization of a catalase-peroxidase and a typical catalase from the bacterium *Klebsiella pneumoniae. Biochim. Biophys. Acta* 1077, 299-307, (1991)
12. Jeffrey, S.W. and G.R. Humphrey. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen* Bd. 167: 191-194, (1975)
13. John F. Schalles et al. Estimation of chlorophyll a from time series measurements of high spectral resolution reflectance in an eutrophic lake. *Journal of Phycology*,Volume 34, Issue 2, pages 383–390, (1998)
14. Karra-Chaabouni M, Pulvin S, Meziani A, Thomas D, Touraud D, KunzW Biooxidation of n-hexanol by alcohol oxidase and catalase in biphasic and micellar systems without solvent. *Biotechnol. Bioeng* .81: 27-32(2003)
15. Kaushik B.D. Laboratory methods for blue- green algae. Associated Publishing Company New Delhi, (1987)
16. Laemmli U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685, (1970)
17. Lohscheider J.N., Strittmatter M, Küpper H, Adamska I. Vertical Distribution of Epibenthic Freshwater Cyanobacterial *Synechococcus* spp. Strains Depends on Their Ability for Photoprotection. *PLoS ONE* 6(5): e20134. doi:10.1371/journal.pone.0020134, (2011)
18. Minkova K.M., Tehernov, A.A., Teharbadjieva M.I.,Fournadjieva S.T., Antova R.E., Busheva MCh. Purification of C-Phycocyanin from *Spirulina(Arthrospora) fusiformis*. *J. Biotechnol*.102: 55-59, (2003).
19. Minu Kesheri, Richa and Rajeshwar P. Sinha. Antioxidants as natural arsenal against multiple stresses in cyanobacteria. *International Journal of Pharma and Bio Sciences*. 2:2, 168-187, (2011)
20. Moreno J., Rodríguez H., Vargas M.A., Rivas J., Guerrero M.G. Nitrogen-fixing cyanobacteria as source of phycobiliprotein pigments. Composition and growth performance of ten filamentous heterocystous. *Journal of Applied Phycology.* 7: 17-23, (1995)
21. Mutsuda M., Ishikawa T., Takeda T.,Shigeoka S. The catalase peroxidase of *Synechococcus* PCC 7942: purification, nucleotide sequence

analysis and expression in *Escherichia coli*, *Biochem. J*. 316:251-257(1996)
22. Obinger, C., Regelsberger, G., Pircher, A., Strasser, G. and Peschek, G. A. Scavenging of superoxide and hydrogen peroxide in blue- green algae (cyanobacteria). *Physiol. Plant*, 140, 693–698, (1998)
23. Padmapriya V. and N. Anand The influence of metals on the antioxidant enzyme,superoxide dismutase, present in the cyanobacterium*, Anabaena variabilis* KÜTZ. *ARPN Journal of Agricultural and Biological Science*, VOL. 5, NO. 2, (2010)
24. Parsons, T.T. and J.D.H. Strickland Discussion of spectrophotometric determination of marine-plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J*. Mar. Res.* 21: 155-163, (1963)
25. Rajendran U.M, Kathirvel E, Anand N. Desiccation-induced Changes in Antioxidant Enzymes, Fatty Acids, and Amino Acids in the Cyanobacterium *Tolypothrix scytonemoides*. *World J Microbiol Biotechnol,* 23:251-257, (2007)
26. Rao, M. V., Paliyath, G. and Ormrod, D. P. Ultraviolet B and ozone- induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol*, 110, 125–136, (1996)
27. Richards, F. A., & Thompson, T. G., The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments*. J. Marine Res*. 11: 156-172, (1952)
28. Rodriguez H., Rivas J., Guerrero M.G., Losada Manuel. Nitrogen- fixing cyanobacterium with a high Phycoerythrin content. *Appl. Environ. Microbiol*. 55: 758-760 (1989)
29. Sartory, D.P. and Grobbelaar. J.U. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia.* 114: 177-187, (1984)
30. Scandalios J.G, Guan L.M, Polidoros A.N. Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Lab. Press, Plainview, N., pp. 343-406, (1997)
31. Sekar S. and Subramanium, G. A method of mass cultivation of the marine cyanobacterium *Phormidium valderiomum* BDU 30501 for the production of blue natural colourant phycocyanin In:G. Subramanium, B.D.Kaushik and G.S. Venkataraman (ed.) Cyanobacterial Biotechnology, Oxford & IBH Publ., Co, New Delhi (1998)
32. Silva HU, Cortinas TI and Ertola RJ. Effect of nutritional factors on the culture of *Nostoc sp.* as a source of phycobiliproteins. *Appl. Microbiol. Biotechnol* 31:293-297, (1989)
33. Stanier, R. Y., R. Kunisawa, M. Mandel, and G. Cohen-Baziere Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev*.35:171-205, (1971)
34. Tel-Or, E., Huflejt, M. E. and Packer, L..Hydrogen peroxide metabolism in cyanobacteria. *Arch Biochem. Biophys.* 246, 396–402, (1986)
35. Tripathi S. N. and Srivastava Pratibha Presence of stable active oxygen scavenging enzymes superoxide dismutase, ascorbate peroxidase and catalase in a desiccation-tolerant cyanobacterium *Lyngbya arboricola* under dry state. Current Science vol.81, No.2, 25, (2001)
36. Tripathi, S. N. and Talpasayi, E. R. S. In Proceedings of National Symposium on Biological Nitrogen Fixation, IARI, New Delhi, pp. 138–149, (1982)
37. UNESCO. Monograph on oceanographic methodology. I. Determination of photosynthetic pigments in sea

water. United Nations Educ., Sci., and Cult. Org., Paris(1966)

38. Weber, C.I., L.A. Fay, G.B. Collins, D.E. Rathke, and J. Tobin. A review of methods for the analysis of chlorophyll in periphyton and plankton of marine and freshwater systems. Ohio State University Sea Grant Program Tech Bull. OHSU-TB-15, (1986)
39. Woodbury W, Spencer A.K, Stahman M.A. An improved procedure using ferricyanide for detecting catalase isozymes. *Anal. Biochem*. 44: 301-305, (1971).
40. Yabuta Y, Fujimura H, Kwak CS, Enomoto T, Watanabe E, Antioxidant activity of the phycoerythrobilin compound formed from a dried Korean purple laver (*Porphyra* sp.) during in vivo digestion, Food Sci Technol Res, 16:347-351, (2010).
41. Zhang Y.M, Chen F. A simple method for efficient separation and purification of C-phycocyanin and allophycocyanin from *Spirulina platensis*. *Biotechnol Tech* 13:601–603(1999)