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Temperature induced physiological and biochemical alterations in the paddy field cyanobacterium *Anabaena doliolum*

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Combustion of fossil fuels and resultant emission of carbon dioxide has led to increased global temperature. Since cyanobacteria are an integral component of the paddy field microflora and contribute to nitrogen fixation, increase in temperature may adversely affect the nitrogen dynamics of the soil. Therefore, to understand the physiological and biochemical response of the mesophilic diazotrophic cyanobacterium *Anabaena doliolum* to elevated temperature, the organism was grown under three temperature regimes 30, 35 and 40°C for 15 days. Exposure of the cyanobacterium to 40°C resulted in severe reduction in growth and cellular constituents as compared to the cells exposed to 35°C. The cyanobacterial cells also showed enhanced production of H_2O_2 and lipid peroxidation products in response to exposure to elevated temperature. Further, we observed increased activity of superoxide dismutase, catalase and peroxidase in *A. doliolum* exposed to elevated temperature. Increase in the temperature resulted in enhanced level of non-enzymatic antioxidants such as carotenoid, proline and ascorbate. Although, the number of heterocysts increased in response to temperature, the nitrogenase activity decreased significantly. The results have demonstrated the sensitivity of the cyanobacterium *A. doliolum* to elevated temperature.

Keywords: Antioxidant enzymes, Biofertilizer, Climate change

The cyanobacteria, commonly known as blue-green-algae are Gram-negative prokaryotes capable of performing oxygenic photosynthesis and nitrogen fixation. These organisms are able to survive on minimum requirement of light, carbon dioxide and water and their occurrence in several agro-eco systems have been discussed[1]. The ability of cyanobacteria to fix atmospheric nitrogen makes them important in any ecosystem[2]. It has been observed that the nitrogen fixing cyanobacteria play an important role in improving the productivity of nitrogen deficient paddy soils. Application of cyanobacteria has been reported to contribute about 20-30 kg N ha^{-1} as well as organic matter to the soil[3].

Human activities coupled with rapid industrialization have resulted in drastic changes in the environment. Mckenzie *et al*.[4] has reported that the global mean temperature change over the 21st century is about 5-fold greater than in the past century. The cyanobacteria have great evolutionary significance and are useful as model for prokaryotic microorganisms to understand the physiological processes. The photosynthetic apparatus of cyanobacteria is similar to higher plants and the ability to fix nitrogen makes them unique and agronomically important. Since cyanobacteria are eco-friendly and important as bioinoculants in agriculture, understanding their response to elevated temperature is important. In cyanobacteria such as *Anacystis nidulans*, elevated temperature stress has been reported to degrade the phycobiliproteins[5]. In *Anabaena doliolum*, induction of antioxidative enzymes in response to elevated temperatures has been observed[6]. Here, we studied the impact of elevated temperature on growth, cellular constituents, nitrogen fixation and antioxidant enzymes in the cyanobacterium *Anabaena doliolum*.

Materials and Methods

The experimental organism *Anabaena doliolum* was provided by Prof. AK Rai, Department of Botany, Banaras Hindu University, Varanasi, Uttar Pradesh, India. *A. doliolum* was routinely maintained in BG-11 medium without added nitrogen. The pH of the medium was adjusted to 7.5 and the cultures were routinely maintained in a culture room at 30°C illuminated with white fluorescent tubes emitting 72 µmol photon $m^{-2}s^{-1}$ PAR (photosynthetically active radiation). Cultures were shaken manually at least two

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to three times a day. For high temperature treatment, the exponentially growing organism was exposed to 35 and 40°C in temperature controlled incubator (BOD) for 15 days and various parameters have been studied.

The dry weight of the cyanobacteria was recorded according to Sorokin[6]. Protein content was estimated by the method of Lowry *et al.*[8] using bovine serum albumin as standard. Total sugar content was estimated by the method of Spiro[9] using glucose as standard. Total chlorophyll content was determined by cold extraction method[10] and the carotenoid content was determined by the method of Jensen[11]. The number of heterocysts per hundred vegetative cells is referred to as heterocyst frequency. For the estimation of nitrogenase acetylene reduction assay was performed according to Stewart *et al.*[12]. The nitrogenase activity was expressed in terms of nmol C_2H_4 mg chlorophyll^{-1}h^{-1}.

Lipid peroxidation was assessed by measuring the total thiobarbituric acid reactive substances and it is expressed as equivalnent of malondialdehyde (MDA) with minor modifications as suggested by Cakmak and Horst[5]. Total peroxide content was estimated according to the protocol given by Sagisaka[13]. Superoxide dismutase activity (SOD) was estimated by recording the decrease in the optical density of formazone made by superoxide radical and nitro-blue tetrazolium dye by the enzyme[14]. Ascorbate peroxidase (APX) was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm[15]. Catalase activity was assayed by measuring the disappearance of H_2O_2 according to Aebi[16]. Proline content was estimated according to the method of Bates *et al.*,[17]. All the experiments have been conducted in triplicate using triplicate samples and the data was further analyzed by Pearson correlation.

Results and Discussion

The growth of the cyanobacterium *A. doliolum* exposed to elevated temperature of 35 and 40°C was recorded in terms of increment in the dry weight. Significant decline in the growth of cyanobacterium was observed due to exposure to elevated temperature (P >0.01, Fig. 1). While, the cyanobacteria showed reduced growth at 35°C, the growth reduction was more pronounced at 40°C. This indicated a differential and general response of the cyanobacterium *A. doliolum* to increase in the ambient temperature and inability to adapt to the changes in the ambient temperature. Mutant strain of *A. doliolum* able to tolerate elevated temperature has been developed[18]. In the cyanobacterium *Spirulina platensis* elevated temperature inhibited the growth and biomass production[19]. Reduced growth of the cyanobacterium *A. doliolum* to elevated temperature is probably a consequence of decrease in photosynthesis. Decrease in photosynthetic efficiency due to high temperature has been observed in cyanobacteria[20]. Further, decrease in the chlorophyll content was also noticed in the cyanobacterium *A. doliolum* due to high temperature (Table. 1). Reduced biosynthesis of chlorophyll as well as its destruction has been reported to be one of the consequences of high temperature in plants[21]. Therefore, reduction in the chlorophyll content due to elevated temperature may lead to reduction in the photosynthetic efficiency and reduction in growth.

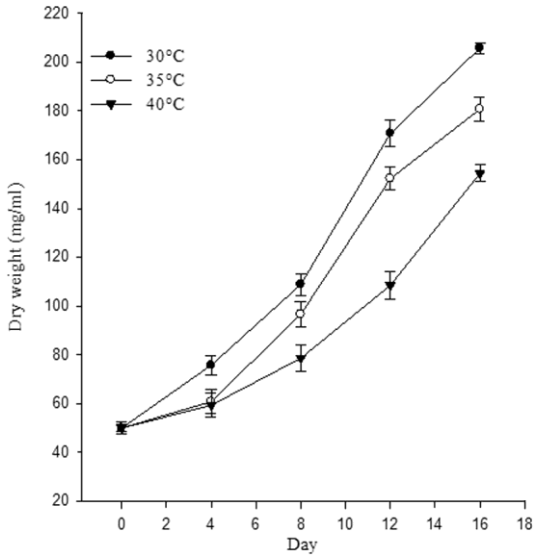

Fig.1 — Growth of *Anabaena doliolum* (dry weight) in response to elevated temperature. [The cyanobacterium was grown in BG 11 medium without nitrogen under standard growth conditions]

Table 1 — Effect of elevated temperature on cellular constituents of the cyanobacterium *Anabaena doliolum*

Temperature (°C)	Total sugar (µg g^{-1} dry wt.)	Protein (µg g^{-1} dry wt.)	Lipid (% dry wt.)	Chlorophyll (µg mg^{-1} dry wt.)
30(Control)	78.5±0.68	162.6±1.13	11.4±0.06	7.8±0.72
35	64.7±0.34	148.4±1.21	14.9±0.13	5.02±0.61
40	39.2±0.28	81.4±1.32	18.6±0.20	1.24±0.19

There are reports on decrease in photosynthesis and reduced growth of the cyanobacterium in response to elevated temperature[22].

While, the protein and sugar content decreased in response to elevated temperature, the lipid content increased (Table. 1). Exposure of the cyanobacterium to elevated temperature resulted in marginal reduction in the protein content (8.2 and 49.9%) whereas the sugar content decreased significantly (17.6 and 51.1%) due to exposure to elevated temperature of 35 and 40°C. One of the classical symptoms associated with heat stress in plants is protein degradation[23]. The observed changes in the pattern of accumulation protein in response to elevated temperature has been supported by the observations of Panyakampol *et al*.[24]. Significant increase in the lipid content was noticed in response to elevated temperature. Stabilization of the membranes is important to maintain the essential physiological processes in response to elevated temperature. Temperature induced increase in the lipid is probably due to the need to stabilize the membranes to maintain the essential physiological processes. Enhancement in the lipid content in microalgae subjected to higher temperature has already been observed[25]. Stabilization of membranes by increasing the degree of fatty acid saturation is thus important during adaptation to temperature stress[26].

The heterocyst frequency of the cyanobacterium decreased due to exposure to elevated temperature whereas significant reduction in the nitrogenase activity was observed (P >0.01, Fig. 2). Defective heterocysts allow oxygen to diffuse in leading to inactivation of the enzyme nitrogenase. Elevated temperature induced changes in the composition of the heterocyst cell envelope in the heterocystous cyanobacteria *Anabaena* sp. strain CCY9613 and *Nostoc* sp. strain CCY9926 in relation to temperature was observed[27]. Furthermore, the process of nitrogen fixation was found to be sensitive to temperature[28,29]. The carotenoid and proline content of the cyanobacterium *A. doliolum* showed a significant increase in response to elevated temperature (Fig. 3A). Increase in carotenoid content by 1.12 and 2.7% when exposed to 35 and 40°C. Carotenoid is an important antioxidant and increase in temperature has resulted in enhanced carotenoid of the cyano-

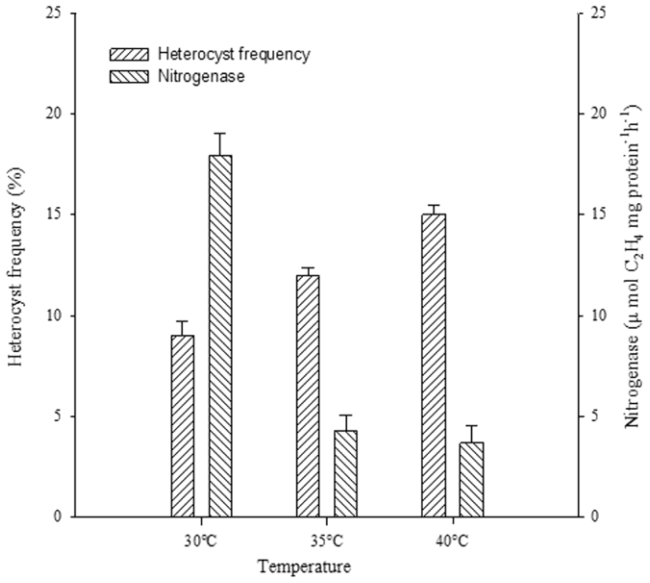

Fig. 2 — Effect of elevated temperature on the heterocyst frequency and nitrogenase activity of *A. doliolum*. [Bars represent mean ± SD of three independent observations]

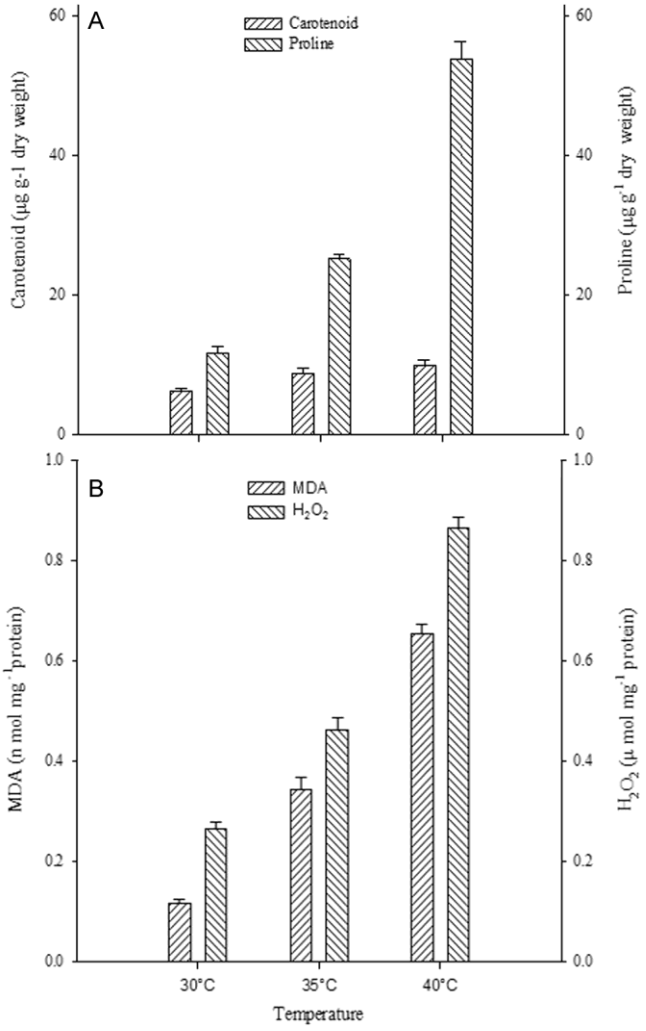

Fig. 3 — (A) Effect of elevated temperature on the carotenoid and proline content; and (B) MDA and H_2O_2 content of *A. doliolum* in response to elevated temperature. [Bars represent mean ± SD of three independent observations]

bacterium *Nostoc muscorum*[30]. Carotenoids play an important role in photoprotection in response to abiotic stress and its role in preventing oxidative damage to membranes have been reported[31]. However, the increase carotenoid content of the cyanobacterium *A. doliolum* exposed to temperature was negligible. The quantum of the pigment and its increase due to the stress condition depends on the species, duration of exposure and inherent ability to tolerate the stress conditions[32]. Therefore, it could be surmised carotenoids have limited role in countering the stress induced by high temperature in the cyanobacterium *A. doliolum*. The proline content increased in the cyanobacterial cells exposed to elevated temperature. Enhanced synthesis of proline in plants conferred significant increase in the heat stress tolerance[33]. Increase in proline content was reported in the mesophilic cyanobacterium *Nostoc muscorum* in response to temperature stress[34]. Hence, increase in the proline accumulation is correlated with the ability to tolerate high temperature.

A. doliolum cells exposed to temperature showed significant increase in the peroxides (H_2O_2) and malondialdehyde (MDA) content (Fig. 3B). Overproduction of ROS in response to heat stress has been observed[35]. Mishra *et al.*[6] observed increase in H_2O_2 content in the cyanobacterium *A. doliolum* exposed to elevated temperature. Exposure to temperature stress results in excessive accumulation of reactive oxygen species[36]. Thus, exposure to elevated temperature increased lipid peroxidation products and resulted in oxidative stress damage in cyanobactria[37]. De Silva & Asaeda[38] correlated increase in the peroxide content with oxidative stress in submerged aquatic macrophytes. Kaushal *et al.*[39] observed that increased levels of MDA due to elevated temperature indicate possible damage to the membranes.

The enzyme super oxide dismutase (SOD) catalyzes the dismutation of superoxide radicals to H_2O_2 and O_2. Further, scavenging of H_2O_2 is done by APX and CAT which prevent the peroxide damage to the cellular constituents by minimizing its accumulation and diffusion across membranes[40]. The antioxidant enzyme activity of the cyanobacterium exposed to temperature was investigated (Fig. 4). In general, stress conditions induced enhanced antioxidant enzyme activity[41]. Elevated temperature enhanced the antioxidant enzyme activity of the cyanobacterium *Microcystis aeruginosa*[42]. Upregulation

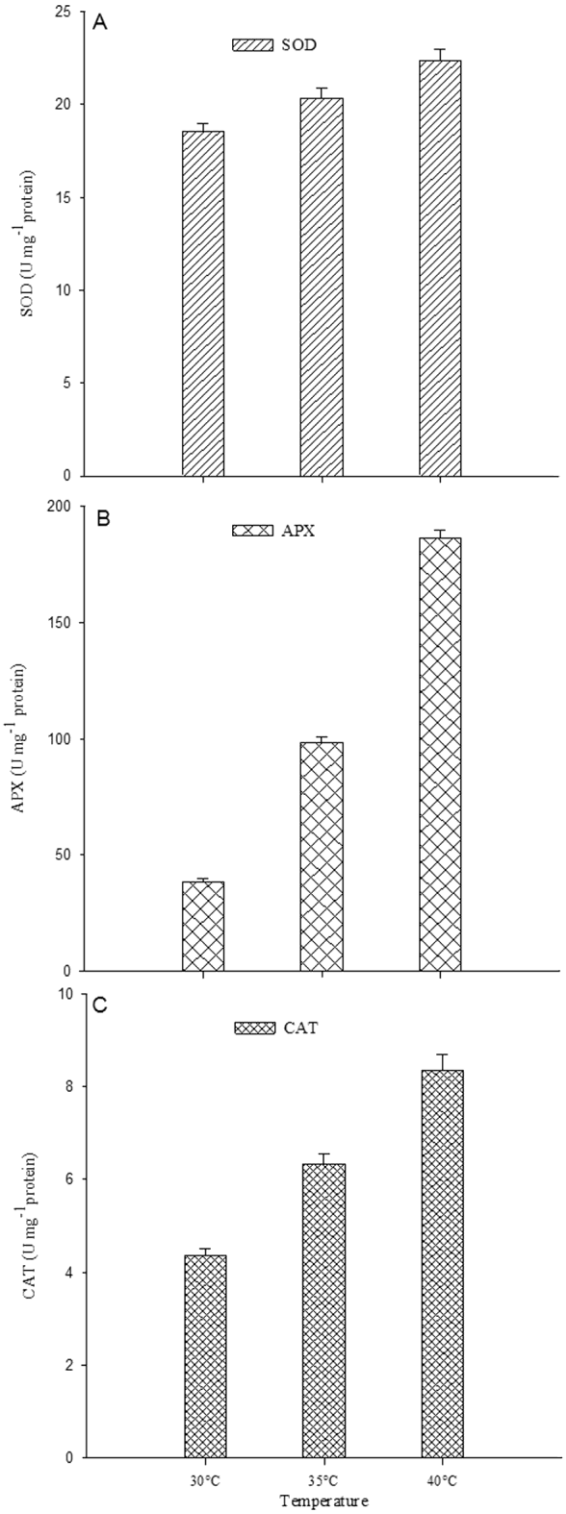

Fig. 4 — Antioxidant enzyme activities (A) SOD; (B) APX; and (C) CAT of *A. doliolum* exposed to elevated temperature. [Bars represent mean ± SD of three independent observations]

of antioxidant enzymes was observed n cyanobacteria to counter oxidative stress[43]. For mitigation of lipid

Table 2 — Correlation coefficient (r) at p value of 0.05 and 0.01 obtained through Pearson method for dry weight content with other parameters in cyanobacterium *Anabaena doliolum* exposed to elevated temperature

Parameter	Dry weight	Chlorophyll	Carotenoid	Total sugar	Protein	Lipid	APX	SOD	CAT	Proline	MDA	H_2O_2
Dry weight	1.0	0.988**	–0.970**	0.876**	0.968**	–0.996**	–0.993**	–0.926**	–0.993**	–0.979**	–0.985**	–0.981**
Chlorophyll	0.988**	1.0	–0.931**	0.935**	0.932**	–0.990**	–0.973**	–0.909**	–0.989**	–0.944**	–0.971**	–0.949**
Carotenoid	–0.970**	–0.931**	1.0	–0.745*	–0.995**	0.971**	0.990**	0.905**	0.961**	0.996**	0.980**	0.996**
Total sugar	0.876**	0.935**	–0.745*	1.0	0.744*	–0.880**	–0.830**	–0.813**	–0.891**	–0.771*	–0.836**	–0.778*
Protein	0.968**	0.932**	–0.995**	0.744*	1.0	–0.969**	–0.987**	–0.886**	–0.958**	–0.994**	–0.975**	–0.992**
Lipid	–0.996**	–0.990**	0.971**	–0.880**	–0.969**	1.0	0.994**	0.931**	0.997**	0.976**	0.986**	0.979**
APX	–0.993**	–0.973**	0.990**	–0.830**	–0.987**	0.994**	1.0	0.923**	0.986**	0.992**	0.994**	0.995**
SOD	–0.926**	–0.909**	0.905**	–0.813**	–0.886**	0.931**	0.923**	1.0	0.930**	0.910**	0.910**	0.909**
CAT	–0.993**	–0.989**	0.961**	–0.891**	–0.958**	0.997**	0.986**	0.930**	1.0	0.968**	0.974**	0.966**
Proline	–0.979**	–0.944**	0.996**	–0.771*	–0.994**	0.976**	0.992**	0.910**	0.968**	1.0	0.985**	0.995**
MDA	–0.985**	–0.971**	0.980**	–0.836**	–0.975**	0.986**	0.994**	0.910**	0.974**	0.985**	1.0	0.990**
H_2O_2	–0.981**	–0.949**	0.996**	–0.778*	–0.992**	0.979**	0.995**	0.909**	0.966**	0.995**	0.990**	1.0

[**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level]

peroxidation induced membrane damage maintenance of high levels of antioxidant activity is required[44]. In *M. aeruginosa* increase in antioxidant enzyme activity was reported in response to elevated temperature[42]. Therefore, increased accumulation of peroxides inhibited the growth in *A. doliolum* despite an increase in the activity of antioxidant enzymes.

Correlation analysis was performed to understand the effect elevated temperature on growth, cellular constituents, H_2O_2, MDA content and antioxidant enzymes (Table 2). Decrease in growth due to high temperature is positively correlated with chlorophyll (r= 0.988), total sugar (r= 0.876) and protein (r= 0.968) content. However, the growth of the cyanobacterium *A. doliolum* in response to temperature is negatively correlated with lipid content (r= -0.996), carotenoids (r= - 0.970), SOD (r= -0.926), APX (r= -0.993), CAT (r= 0.993), H_2O_2 (r=-0.981), MDA (r=-0.985) and proline (r= -0.979). These results further indicate the adverse impact of elevated temperature on the cellular constituents, such as chlorophyll, total sugar and protein content of the cyanobacterium *A. doliolum.*

The present study has demonstrated the sensitivity of the cyanobacterium *Anabaena doliolum* to elevated temperature. Exposure to elevated temperature may affect the nitrogen metabolism in cyanobacteria and alter the dynamics of nitrogen cycling in the ecosystem. *A. doliolum* is an important nitrogen fixing cyanobacterium commonly found in rice paddy fields and it helps to maintain the nitrogen dynamics. Adverse impact of elevated temperature may thus increase our dependence on chemical nitrogen fertilizers to a certain extent and leads to global climate change.

Conclusion

Exposure of the cyanobacterium to 40°C temperature resulted in severe reduction in growth, cellular constituents and nitrogen fixation. However, the activity of enzymatic and non-enzymatic antioxidant enzymes enhanced with corresponding increase in the accumulation of peroxides and lipid peroxidation products. From the results it appears that the cyanobacterium *Anbaena doliolum* is sensitive to elevated temperature.

Conflict of Interest

None

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References

1 Castenholz RW, Wilmotte A, Herdman M, Rippka R, Waterbury JB, Iteman I & Hoffmann L & Phylum BX. Cyanobacteria. In: *Bergey's Manual of Systematic Bacteriology*, 1, (Eds. Boone, DR & Castenholz RW. Springer, New York), 2001, 473.

2 Bothe H, Tripp HJ & Zehr JP, Unicellular cyanobacteria with a new mode of life: the lack of photosynthetic oxygen evolution allows nitrogen fixation to proceed. *Arch Microbiol*, 192 (2010) 783.

3 Issa AA, Abdalla MH & Ohyama T, Nitrogen fixing cyanobacteria: future prospect. In: *Advances in Biology and Ecology of Nitrogen Fixation*, (Ed. T Ohyama: Intechopen, London, UK), 2014.

4 Mckenzie RL, Aucam PJ, Bais AF, Bjorn LO & Iiyas M, Changes in biologically active ultraviolet radiation reaching the earth surface. *Photochem Photobiol Sci*, 6 (2007) 218.

5 Cakmak I & Horst WJ, Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol Plant*, 83 (1991) 463.

6 Mishra Y, Bhargava P & Rai LC, Differential induction of enzymes and antioxidants of the antioxidative defense system in *Anabaena doliolum* exposed to heat stress. *J Ther Biol*, 30 (2005) 524.

7 Sorokin C, Dry weight packed cell volume and optical density. In: *Hand Book of Phycological Methods. Culture Methods and Growth Measurements*. (Ed. Stein JR: Cambridge University Press, Cambridge), 1973, 321.

8 Lowry OH, Rosebrough NJ, Farr AL & Randall RJ, Protein measurement with the Folin-Phenol reagents. *J Biol Chem*, 193 (1951) 265.

9 Spiro RA, Analysis of sugars found in glycoproteins. *Methods Enzymol*, 8 (1966) 3.

10 McKinney, Absorption of light by chlorophyll solutions. *J Biol Chem*, 140 (1941) 315.

11 Jensen A, Chlorophylls and Carotenoids, *In*: *Phycological Methods: Physiological and Biochemical Methods*, (Eds. Hellebust JA & Craige JS; Cambridge University Press, Cambridge), 1978, 59.

12 Stewart WDP, Fitzgerald GP & Burris RH, Acetylene reduction by nitrogen fixing blue green algae. *Arch Microbiol*, 62 (1968) 336.

13 Sagisaka S, "The occurrence of peroxide in a perennial plant, *Populus gelrica*. *Plant Physiol*, 57 (1976) 308.

14 Dhindsa RS, Plumb Dhindsa P & Thorpe TA, Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot*, 32 (1981) 93.

15 Nakano Y & Asada K, Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*, 22 (1981) 867.

16 Aebi H, Catalase *in vitro*. *Meth Enzymol*, 105 (1984) 121.

17 Bates LS, Wadern RP & Teare ID, Rapid estimation of free proline for water stress determination. *Plant Soil*, 39 (1973) 205.

18 Reddy YP, Yadav RK, Tripathi K & Abraham G, Isolation and characterization of high temperature tolerant mutant from the cyanobacterium *Anabaena doliolum*. *J Basic Microbiol*, (2018) 1. DOI: 10.1002/jobm.201800447

19 Chaiklahan R, Khonsarn N, Chirasuwan N, Ruengjit-chatchawalya M, Bunnag, B & Tanticharoen M, Response of *Spirulina platensis* C1 to high temperature and high light intensity. *Kasetsart J* (*Nat Sci*), 41 (2007) 123.

20 Lan SB, Wu L, Zhang DL & Hu CX, Composition of photosynthetic organisms and diurnal changes of photosynthetic efficiency in algae and moss crusts. *Plant Soil*, 351 (2012) 325.

21 Reda F & Mandoura HMH, Response of enzymes activities, photosynthetic pigments, proline to low or high temperature stressed wheat plant (*Triticum aestivum* L.) in the presence or absence of exogenous proline or cysteine. *Int J Acad Res*, 3 (2011) 108.

22 Mishra Y, Chaurasia N & Rai LC, Heat pretreatment alleviates UV-B toxicity in the cyanobacterium *Anabaena doliolum*: A proteomic analysis of cross tolerance. *Photochem Photobiol*, 85 (2009) 824.

23 Savchenko G, Klyuchareva E, Abramchik L & Serdyuchenko E, Effect of periodic heat shock on the innermembrane system of etioplasts. *Russ J Plant Physiol*, 49 (2002) 349.

24 Panyakampol J, Cheevadhanarak S, Sutheeworapong S, Chaijaruwanich J, Senachak J, Siangdung W & Paithoonrangsarid K, Physiological and transcriptional responses to high temperature in *Arthrospira platensis* (Spirulina) C1. *Plant Cell Physiol*, 56 (2014) 481.

25 Xin L, Hongying H & Yuping Z, Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. *Bioresour Technol*, 102 (2011) 3098.

26 Balogi Z, Török Z, Balogh G, Jósvay K, Shigapova N, Vierling E, Vígh L & Horváth I, "Heat shock lipid" in cyanobacteria during heat/light-acclimation. *Arch Biochem Biophys*, 436 (2005) 346.

27 Bauersachs T, Stal LJ, Grego M & Schwark L, Temperature induced changes in the heterocyst glycolipid composition of N_2 fixing heterocystous cyanobacteria. *Org Geochem*, 69 (2014) 98.

28 Rajaram H & Apte SK, Heat shock response and its contribution to thermotolerance of the nitrogen-fixing cyanobacterium *Anabaena* sp. strain L-31. *Arch Microbiol*, 179 (2003) 423.

29 Chaurasia AK & Apte SK, Overexpression of the groESL operon enhances the heat and salinity stress tolerance of the nitrogen fixing cyanobacterium *Anabaena* sp. strain PCC7120. *Appl Environ Microbiol*, 75 (2009) 6008.

30 Kuhne S, Michael L, Sarah F, Kai M & Roland U, Characterization of terrestrial cyanobacteria to increase process efficiency in low energy consuming production processes. *Sust Chem Proc*, 1 (2013).

31 Meiri D, Tazat K, Cohen-Peer R, Farchi-Pisanty O, Aviezer-Hagai K, Avni A & Breiman A, Involvement of *Arabidopsis* ROF2 (FKBP65) in thermotolerance. *Plant Mol Biol*, 72 (2010) 191.

32 Ashraf M & Harris PJC, Photosynthesis under stressful environments: An overview**.** *Photosynthetica*, 51:2 (2013) 163.

33 Priyanka B, Sekhar K, Reddy VD & Rao KV, Expression of pigeonpea hybridproline-rich protein encoding gene (CcHyPRP) in yeast and *Arabidopsis* affords multiple abiotic stress tolerance. *Plant Biotechnol J*, 8 (2010) 76.

34 Singh S, Kate BN & Banerjee UC, Bioactive compounds from cyanobacteria and microalgae: An overview. *Crit Rev Biotech*, 25 (2005) 73.

35 Wahid A, Gelani S, Ashraf M & Foolad M, Heat tolerance in plants: an overview. *Environ Exp Bot*, 61 (2007) 199.

36 Foyer CH, Lelandais M & Kunert KJ, Photooxidative stress in plants. *Physiol Plant*, 92 (1994) 696.

37 He YY & Häder DP, Reactive oxygen species and UV-B: effect on cyanobacteria. *Photochem Photobiol Sci*, 1 (2002) 729.

38 De Silva HCC & Asaeda T, Effects of heat stress on growth, photosynthetic pigments, oxidative damage and competitive

capacity of three submerged macrophytes. *J Plant Int*, 12 (2017) 228.

39 Kaushal N, Gupta K, Bhandhari K, Kumar S, Thakur P & Nayyar, Proline induces heat tolerance in chickpea (*Cicer arietinum* L.) plants by protecting vital enzymes of carbon and antioxidative metabolism. *Physiol Mol Biol Plants*,17 (2011) 203

40 Mittler R, Vanderauwera S, Gollery M & Breusegem FV, Reactive oxygen gene network of plants. *Trends Plant Sci*, 9 (2004) 490.

41 Jahantigh O, Najafi F, Badi HN, Khavari-Nejad RA & Sanjarian F, Changes in antioxidant enzymes activities And proline, total phenol and anthocyanine contents in *Hyssopus Officinalis* L. plants under salt stress. *Acta Biol Hung*, 67 (2016) 195.

42 Han W, Jing Y & Ting L, Compensatory Growth in *Microcystis aeruginosa* after moderate high temperature exposure. *J Limnol*, 74 (2015).

43 Srivastava AK, Bhargava P, Thapar R & Rai LC, Salinity-induced physiological and proteomic changes in *Anabaena doliolum*. *Environ Exp Bot*, 64 (2008) 49.

44 Xu S, Li J, Zhang X, Wei H & Cui L, Effect of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool season turf grass species under heat stress. *Environ Exp Bot*, 56 (2006) 274.