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Impact of osmotic stress and temperature on pigments and proteins of *Anabaena* strains

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Abstract

A study on *Anabaena* strains was carried out to investigate the effect of combined stress of polyethylene glycol 6000 (0,-5 bar and -7 bar) and temperature (30°C and 45 °C) on photosynthetic pigments (chlorophyll a, carotenoids, phycobilins) and total proteins as stress metabolites. The selected strains, *A. oryzae* and *A. ellipsospora* were sensitive to osmotic stress at ambient temperature of 30 °C and increase in the temperature to 45 °C was harmful to the growth of *Anabaena* strains. Chlorophyll a contents decreased at 30 °C and -7 bar pressure from 8.868 to 0.710 µg ml^{-1} and 4.360 to 0.220 µg ml^{-1} in *A. oryzae* and in *A. ellipsospora*, respectively and at -7 bar osmotic stress and 45 °C temperature, decrease in Chl a content of *A.oryzae* was 92.9%, however *A. ellipsospora* was highly sensitive and could not survive under these conditions. Carotenoids and phycobilins also showed decreasing trends with increase in temperature and osmotic potential. Moreover, combined stress adversely depleted the cellular activities leading to a marked decrease in total protein contents of the cell. *A.oryzae* and *A.ellipsospora* showed varying tolerance potential to osmotic and temperature stresses. The results indicated that *A. ellipsospora* was more sensitive towards these stresses in comparison to *A.oryzae*.

Key words

Anabaena strains, Osmotic stress, Photosynthetic pigments

Introduction

The environment is fast becoming increasingly stressful. Studies on the response of microbes and plants to environmental stressors, such as salinity, osmotic stress, drought, heat, and so forth, form a thrust area of contemporary biological research. Cyanobacteria are a major group of bacteria that occur throughout the world (Al-kahtani and Fathi, 2008). Cyanobacteria exhibit a close phylogenetic relationship with plant chloroplast and therefore, are regarded as most appropriate model system for studying plant responses to various stresses (Apte, 2001). The cosmopolitan distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as heat, cold, desiccation, salinity, nitrogen starvation, photo-oxidation, and osmotic stress etc. Cells that are exposed to stresses undergo changes in their metabolism in order to adapt with changes in their environment. Stress changes the morphological, physiological and biochemical responses and adversely affects the growth and development of cells (Amirjani, 2011). Stress allevation in cyanobacteria has been known to synthesize variety of proteins (Karthikeyan and Gopalaswamy, 2009). Under stress conditions cyanobacterial pigments, i.e. chlorophyll a, carotenoids and phycocyanin are adversely affected. Furthermore, cells produce more peroxide radicals (Stahl and Sies, 2005). The PEG is a polymer which is produced in a wide range of molecular weights. The PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce water deficit in a relatively controlled manner (Lagerweff *et al*., 1961). In general, water plays a crucial role for all metabolic activities and cellular dehydration can inhibit photosynthesis (Gray *et al*., 2007).

The study presented here investigates the effect of osmotic stress and temperature on the photosynthetic pigment and total protein content of *Anabaena* strains.

Materials and Methods

Experimental set-up : The cyanobacterial isolates *A. oryzae* and *A. ellipsospora* obtained from the Department of Microbiology, M. D. S. University, Ajmer were cultured on BG-11 medium (Stainer *et al*., 1971) without nitrogen supplements. Exponentially grown cyanobacterial cells were used throughout the experiment. Each experiment was conducted in replicates of three and their ± SD values were calculated.

A. oryzae and *A. ellipsospora* strains were inoculated on different concentration of PEG 6000 under standard conditions to generate osmotic water potential of 0, -5 and -7 bar (Kaufmann and Eckard, 1970) and were kept at 45 ^{0}C in BOD incubator for induction of thermal stress and at normal room temperature (30 ^{0}C) in growth room for 20 days.

Parameters : Before inoculation and after 20 days, chlorophyll a, phycobilins, carotenoids and total protein were estimated. Chlorophyll a was determined following the method of Porra *et al*. (1989). The methanol extraction supernatant was estimated for cellular Chlorophyll a content. Carotenoids was extracted in 85% acetone and determined spectrophotometrically by measuring the absorbance at 480 nm (Jensen, 1978). Phycobilins was extracted in 0.1 M solution of K_2HPO_4 and KH_2PO_4 and after repeated freezing and thawing, was measured at 615 and 652 nm, respectively (Bennett and Bogorad, 1973). Total protein estimation was done by the method of Lowry *et al*. (1951).

Results and Discussion

The *A. oryzae* and *A. ellipsospora* showed inhibitory growth response against the osmotic potential and increase in temperature. Osmotic stress of -7 bar at 30ºC reduced Chl a by 91.99 % in *A. oryzae* (Table 1) and by 94.95 % in *A. ellipsospora,* (Table 2). Similar decreasing trends in Chl a contents were observed at 45ºC in *A. oryzae* (Table 1) and *A. ellipsospora* (Table 2). Carotenoid content in both the strains decreased from -5 to -7 bar, but contents of carotenoid were higher at 30 ºC as compared to 45 ºC with different PEG concentration. The lowest carotenoid content was 0.0013 µg ml $^{-1}$ in *A. ellipsosopra* at -7 bar and 45 ºC temperature (Table 2). Moreover, the phycobilins also were found to decrease significantly with respect to increasing concentration of PEG and temperature. The -7 bar osmotic pressure at 30 ºC and at 45 ^{0}C reduced phycobilins content by 91.30 % and by 94.59 % in *A. oryzae* (Table 1) and by 97.14 and 100% in *A. ellipsospora* (Table 2). The reduction in Chl a, carotenoids and phycobilins content may be ascribed to the inhibition of pigment synthesis. Sundaram and Soumya (2011) also reported similar observation while studying the physiological and biochemical alteration in cyanobacterium under organic stress. The reduction in chlorophyll content due to different stresses may be inhibition of δ-aminolevulinic acid dehydrogenase and protochlorophyllide reductase (Ouzounido, 1995). In cyanobacteria, phycobiliproteins (PBP_S) that are attached to the stromal surface of thyalakoid membranes serve as the primary light-harvesting antenna for PS2. The composition and function of PBP_S in cyanobacteria changed in response to stress conditions.Total protein content also exhibited decreasing trend with increasing osmotic pressure

Table 1 : Effect of osmotic stress and temperature on photosynthetic pigments and protein content content of *Anabaena* strain

Osmotic potential (bar)	Chlorophyll		Carotenoid		Phycobilin		Protein	
	30ºC	45ºC	30ºC	45ºC	30ºC	45ºC	30ºC	45ºC
Anabaena oryzae								
0	8.868±0.042	2.665±0.021	0.0263±0.028	0.0168±0.031	0.115±0.032	0.037±0.014	100.936±0.025	81.98±0.075
-5	1.772±0.035	0.459±0.015	0.0047±0.011	0.0026±0.015	0.049±0.021	0.009±0.012	80.795±0.021	64.67±0.014
	(-80.05)	(-82.77)	(-82.14)	(-84.52)	(-57.39)	(-75.67)	(-19.95)	(-21.18)
-7	0.710±0.011	0.187±0.012	0.0036±0.013	0.0017±0.011	0.010±0.012	0.002±0.01	68.825±0.012	54.89±0.011
	(-91.99)	(-92.98)	(-86.31)	(-89.88)	(-91.30)	(-94.59)	(-31.81)	(-33.04)
Anabaena ellipsospora								
0	4.360±0.038	4.260±0.038	0.0259±0.038	0.0189±0.031	0.175±0.021	0.095±0.023	222.34±0.081	134.43±0.022
-5	0.651±0.031	0.421±0.018	0.0027±0.016	0.0018±0.011	0.041±0.018	0.012±0.011	79.69±0.27	46.71±0.015
	(-85.06)	(-90.11)	(-89.57)	(-90.47)	(-76.57)	(-87.36)	(-64.15)	(-65.25)
-7	0.220±0.016	_	0.0019±0.013	0.0013±0.012	0.005±0.011	_	60.35±0.021	34.92±0.012
	(-94.95)		(-92.66)	(-93.12)	(-97.14)		(-72.85)	(-74.02)

Values are mean of 3 replicates ± SD. Values in parentheses show % decrease

and temperature. Protein content declined radialy when treated with different PEG concentration. As shown in Table 1, the reduction in *A. oryzae* at -7 bar osmotic pressure was 31.81 (at 30 ^{0}C) and 33.04% (at 45 ^{0}C). However, in *A. ellipsospora* reduction at -7 bar osmotic pressure was 72.85 (at 30 ^{0}C) and 74.02% (at 45 ^{0}C). Hiremath and Mathad (2010) also observed similar depletion in protein and chlorophyll contents while working on impact of salinity on the physiological and biochemical traits of *Chlorella vulgaris*. Bhadauriya *et al.* (2007) also reported the inhibition in growth in response to a high concentration of NaCl, which might be due to inhibition of photosynthetic and respiratory systems. It has also been reported that cyanobacterium cultured with a higher salt concentration had lower chlorophyll and protein contents. Thus, NaCl stress adversely limits the growth and protein synthesis in *A. cylindrica* because of either its osmotic or ionic impact on the cell metabolism and consequently causing other physiological and biochemical imbalances. These results are also supported by the earlier findings of Moisander *et al*. (2002) in eustarine planktonic cyanobacteria.

The present study showed that osmotic and temperature stress generated significant reduction in photosynthetic pigments and total protein contents in both the *Anabaena* strains. The percent reduction in all the parameter was higher in *A.ellipsospora* indicates higher sensitivity than *A.oryzae*.

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