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ENHANCED PIGMENT PRODUCTION IN SELECTED CYANOBACTERIA THROUGH CULTURAL MANIPULATIONS

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Four cyanobacterial genera namely *Oscillatoria*, *Lyngbya*, *Anabaena* and *Microchaete* isolated from paddy fields of Uttar Pradesh were used in the study. Increase in light intensity enhanced pigment production in general; however the effect of varying temperature and CO_2 concentration was variable for pigment production. Production of pigments under optimised conditions in selected cyanobacterial genera may be used in biotechnological applications.

Key words: Cyanobacteria, pigments, enhancements, cultural manipulations.

The cyanobacteria are gram negative prokaryotes showing oxygenic photosynthesis. These organisms have a potential to produce light harvesting pigments namely chlorophyll-a, carotenoids and phycobiliproteins. Chlorophylls function as pivotal pigments with carotenoids and phycobilins as accessory pigments. Chlorophylls are key compounds for trapping light energy for photosysntesis, thus, their quantitative determination is of great importance in studies of photosysnthesis and biomass production (Hertzberg *et al.* 1971). Accessory pigments confer extended ability to harvest light for photosynthesis and in some cases protection from UV and other light induced cell damage. The association of carotenoids with chlorophylls prevents the formation of highly reactive singlet oxygen radicals that would otherwise cause irreparable damage to lipids, proteins and other molecules (Bartley and Scolnick 1995). A wide variety of carotenoids are found in these including α, β-carotene, echinenone, zeaxanthin, myxoxanthophyll and oscillaxanthin (Clayton and King 1990). Phycobilins are water-soluble pigments located on the periphery of the thylakoid membrane (Humm and Wick 1980).

MATERIALS AND METHODS

Cyanobacterial cultures namely *Oscillatoria, Lyngbya, Anabaena* and *Microchaete* were isolated from paddy field soils of Rohilkhand region of India following standard enrichment culture technique. Their unialgal population was raised in nitrogen free for heterocystous and nitrogen supplemented for non-heterocystous genera in BG-11 medium (Stanier *et al.* 1995) in a culture room at 52-55μmole photon/m^2/s light intensity with 16/8 light and dark period and 28± 2^0C temperature. Cyanobacterial cultures were incubated under varying temperatures (25, 30 and 35^{0}C), light intensity (4, 5, 6 KLux), and CO_2 incubation (350, 550 and 750ppm) separately in different growth chambers of National Phytotron Facilities of IARI, New Delhi. In addition, the effect of $ZnSO_4$ (0.5 mg/L) and glutamine (1mM) either alone or in combination was also studied on the pigment production.

Known volume of exponentially growing cultures from different treatments was centrifuged and pellet was used for the determination of photosynthetic pigments

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namely chlorophyll, carotenoids and phycobiliproteins (Phycocyanin, allophycocyanin and phycoerythrin). Cultures were grown in conical flasks with initial volume of 100 mL capacity in triplicates under varying cultures conditions. Growth was measured in terms of dry weight and chlorophyll was extracted in methanol and absorbance was read at 650nm and 665nm for estimation of chlorophyll (McKinney *et al.* 1941). Carotenoids were extracted in acetone and analysed by taking absorbance at 450nm (Jensen *et al.* 1978). Phycobilins were extracted in phosphate buffer and quantified from the absorbance read at 562, 615 and 652nm (Bennet and Bogard 1973).

Studies were undertaken to assess the influence of environmental variables (Light, temperature and CO_2 concentration) and nutrient supplementation on pigment profile in selected cyanobacterial genera namely *Oscillatoria, Lyngbya, Anabaena* and *Microchaete.* The influence of environmental variables and nutrient supplementation on pigment production on cyanobacteria was found to be variable in selected genera. Padgett and Krogmann (1987) described simple procedures for their purification from Microcystis aeruginosa. Their content is affected by growth conditions like light intensity and quality, as well as nitrogen supply (Boussiba and Richmond 1979).

RESULTS AND DISCUSSION

Table 1: Effect of glutamine (1 mM) and $ZnSO_4$(0.5mg/L) on pigments (µg/g dry weight) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean±SD)

Chlorophyll				
Strain	Control	Glutamine	$ZnSO_4$	Glutamine+ $ZnSO_4$
Oscillatoria	506±20.05	560.2±26.50	181.6±57.71	1500.0±51.72
Lyngbya	379.6±23.56	314.6±17.19	192.6±36.55	99.39±34.59
Anabaena	455.1±22.92	-	-	-
Microchaete	142.6±2.07	-	-	-
Carotenoids				
Strain	Control	Glutamine	$ZnSO_4$	Glutamine+ $ZnSO_4$
Oscillatoria	58±3.32	38±3.73	19±2.11	35±3.67
Lyngbya	41±2.09	37±2.58	17±1.84	13±1.11
Anabaena	1544±50.56	-	-	-
Microchaete	998±33.67	-	-	-
Phycocyanin				
Strain	Control	Glutamine	$ZnSO_4$	Glutamine+ $ZnSO_4$
Oscillatoria	1420±23.6	2064±65.71	767±29.56	7625±330.7

Chlorophyll content of *Oscillatoria* and *Microchaete* enhanced with varying temperatures of 25^{0}C, 30^{0}C and 35^{0}C. In *Lyngbya* and *Anabaena*, the increase in temperature from 25^{0}C to 30^{0}C enhanced chlorophyll content followed by a reduction at 35^{0}C. Carotenoids enhanced with increase in

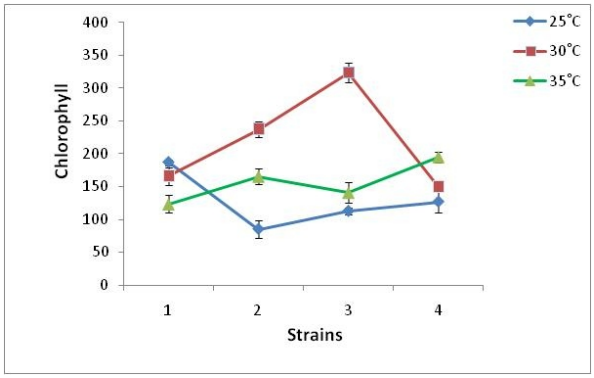
Lyngbya	1286±19.78	4955±263.3	2635±199.7	1244±79.87
Anabaena	954±17.56	-	-	-
Microchaete	865±21.56	-	-	-
Allophycocyanin				
Strain	**Control**	**Glutamine**	**$ZnSO_4$**	**Glutamine+ $ZnSO_4$**
Oscillatoria	1058±35.67	1684±40.89	402±15.91	3441±108.6
Lyngbya	939±33.89	1612±36.09	762±28.33	372±13.75
Anabaena	999.7±16.74	-	-	-
Microchaete	1009±21.73	-	-	-
Phycoerythrin				
Strain	**Control**	**Glutamine**	**$ZnSO_4$**	**Glutamine+ $ZnSO_4$**
Oscillatoria	422±21.34	944±45.23	393±23.42	6214±209.8
Lyngbya	88±4.98	4041±100.9	2164±55.82	944±45.73
Anabaena	4309±119.7	-	-	-
Microchaete	1562±89.91	-	-	-
Total phycobilins				
Strain	**Control**	**Glutamine**	**$ZnSO_4$**	**Glutamine+ $ZnSO_4$**
Oscillatoria	2901	4693	1563	17281
Lyngbya	2314	10609	5562	2561
Anabaena	2385	-	-	-
Microchaete	3437	-	-	-

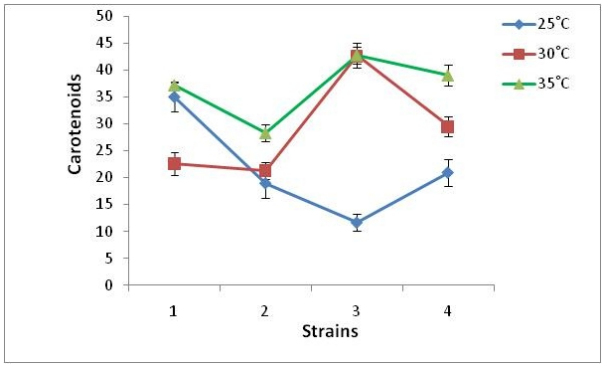
temperature in *Lyngbya*, *Anabaena* and *Microchaete*, however, 30^0C was inhibitory to the carotenoids in *Oscillatoria* as compared to 25^0C. A preparation of *Spirulina* chlorophyll in a mixture containing iron oxide and higher alcohol was patented as a strong deodorant (Yamaguchi 1981). Carotenoids have commercial applications as food colouring and feed additives to enhance flesh colour of salmonoid fish, as well as the colour of the egg yolk. These also improve the health and fertility of cattle (Borowitzka 1988). However increase in temperature of 35^0C showed an enhancement in the level of carotenoids. The effect of varying temperatures on phycocyanins was variable. The highest phycocyanin content was recorded at 30^0C in *Oscillatoria, Lyngbya* and *Microchaete*. On the other hand 25^0C was optimum for enhanced production of phycocyanin in *Anabaena*. Allophycocyanin showed a reduction with enhanced temperature in *Oscillatoria* and variable response in other three cyanobacteria. The effect of temperature on phycoerythrin content was also variable in four cyanobacterial genera. All naturally occurring cyanobacteria are found to produce PC (Bryant 1982). In the Anabaena PCC7120 only a single set of PC related genes have been identified which are constitutive in nature (Sidler 1994). In contrast, PE is not always found in all cyanobacteria living in either fresh water or in soil or marine environments (Bryant 1982). Increase in light intensity from control (4 Klux)

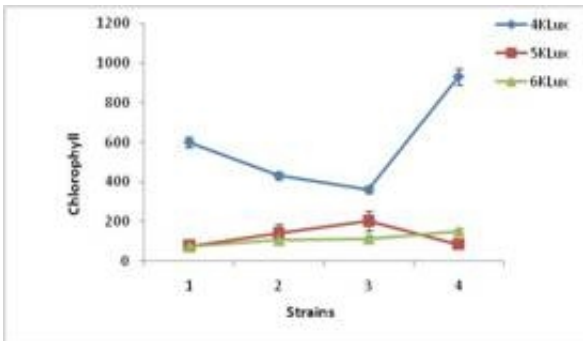
till 6 decreased chlorophyll content significantly in *Oscillatoria, Lyngbya* and *Anabaena* while in *Microchaete* the light intensity of 5 is inhibitory. Carotenoids also showed a significant reduction with increase in light intensity. The effect of varying light intensities on phycobilins was variable. Total phycobilins exhibited a varied response towards increase in light intensity as well as temperature. The effect of varied levels of CO_2 depicted variable pattern in terms of chlorophyll, carotenoids and phycobilins. Incubation of *Oscillatoria* at 550 ppm CO_2 level enhanced chlorophyll content as compared to the incubation at 350 ppm. The chlorophyll content reduced significantly at 750 ppm of CO_2 incubation in *Oscillatoria*. In *Lyngbya* enhanced CO_2 levels increased chlorophyll content and in *Anabaena* and *Microchaete* 750 ppm was optimum. Incubation of cyanobacterial cultures at 750 ppm enhanced carotenoids as compared to 350 ppm. On the other hand incubation at 550 ppm was inhibitory. In *Lyngbya* 550 ppm CO_2 appeared to be optimum. Enhanced levels of CO_2 exhibited an inhibitory influence on phycocyanin in all four genera and allophycocyanin in *Oscillatoria* and *Lyngbya*. 350 ppm CO_2 was optimum for allophycocyanin production in *Anabaena* and *Microchaete*. The effect of varying levels of CO_2 on phycoerythrin content was variable with the highest production recorded at 350 ppm CO_2 in all the four genera examined. Increased in CO_2 concentration decreased total phycobilins in *Oscillatoria*, *Lyngbya* and *Anabaena*. There was a significant reduction in total phycobilins at 550 ppm CO_2 as compared to 350 ppm CO_2 followed by an increase at 750 ppm of CO_2 incubation in *Microchaete*. The ranking of strains of PC, APC and PE content exhibits there potential utilisation as colouring agents, phycoflour probes or as additives in a range of cosmetics and pharmaceutical products (MacColl and Guard-Frair 1987).

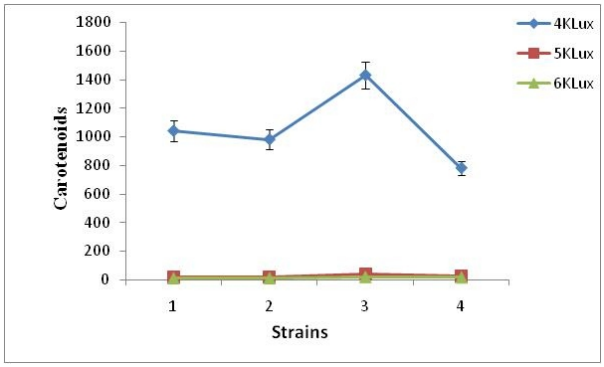
Studies conducted on the role of glutamine, $ZnSO_4$ either taken alone or in combination showed that *Anabaena* and *Microchaete* did not grow in glutamine and $ZnSO_4$ supplemented medium. On the other hand, the chlorophyll enhanced significantly with glutamine and $ZnSO_4$ as compared to control in *Oscillatoria* and *Lyngbya*. When glutamine and $ZnSO_4$ were taken together, there was a marked enhancement in chlorophyll content of *Oscillatoria*. In *Lyngbya*, glutamine supplemented medium showed highest chlorophyll followed by $ZnSO_4$ supplemented medium and glutamine together with $ZnSO_4$ supplemented medium. Chlorophyll was significantly lower under control grown cultures in standard BG-11 medium. Maximum carotenoids were recorded in control grown cultures of *Oscillatoria* and *Lyngbya*. Supplementation of medium with glutamine and $ZnSO_4$ either alone or in combination showed an inhibitory effect on the levels of carotenoids in these two genera. Glutamine supplementation either alone or in combination with $ZnSO_4$ enhanced phycocyanin in *Oscillatoria* in comparison to control. $ZnSO_4$ had an inhibitory effect on phycocyanin of *Oscillatoria*. On the other hand glutamine and $ZnSO_4$ supplementation in the growing medium separately showed an enhanced production of phycocyanin in *Lyngbya* while these two together influenced phycocyanin content as compared to control in *Lyngbya*.

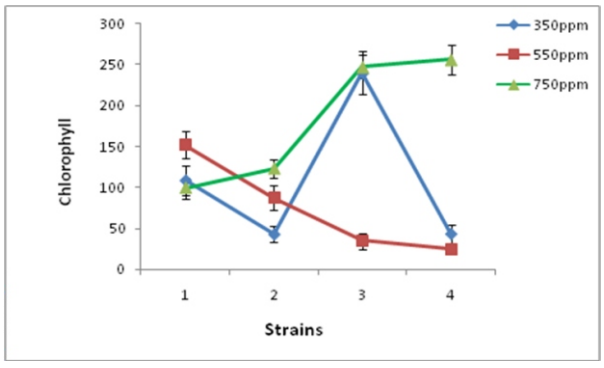
Allophycocyanin was reduced with $ZnSO_4$ in growing medium as compared to control in *Oscillatoria*, however, this together with glutamine enhanced allophycocyanin content. Glutamine alone also exhibited positive influence on allophycocyanin content in











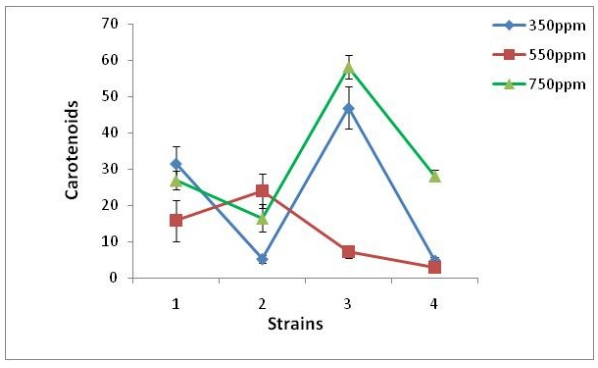


Figure1:Comparative chlorophyll andcarotenoids(µg/gdryweight)atdifferenttemperatures(25^{0}C, 30^{0}C,and35^{0}C),lightintensities(4KLux,5KLuxand6KLux)andCO_2concentrations(350ppm,550ppm and750ppm)inselectedcyanobacterialgeneraisolatedfrompaddyfieldsofUttarPradesh(Eachvalueis mean±SD);1-*Oscillatoria*,2-*Lyngbya*,3-*Anabaena* and 4-*Microchaete*.

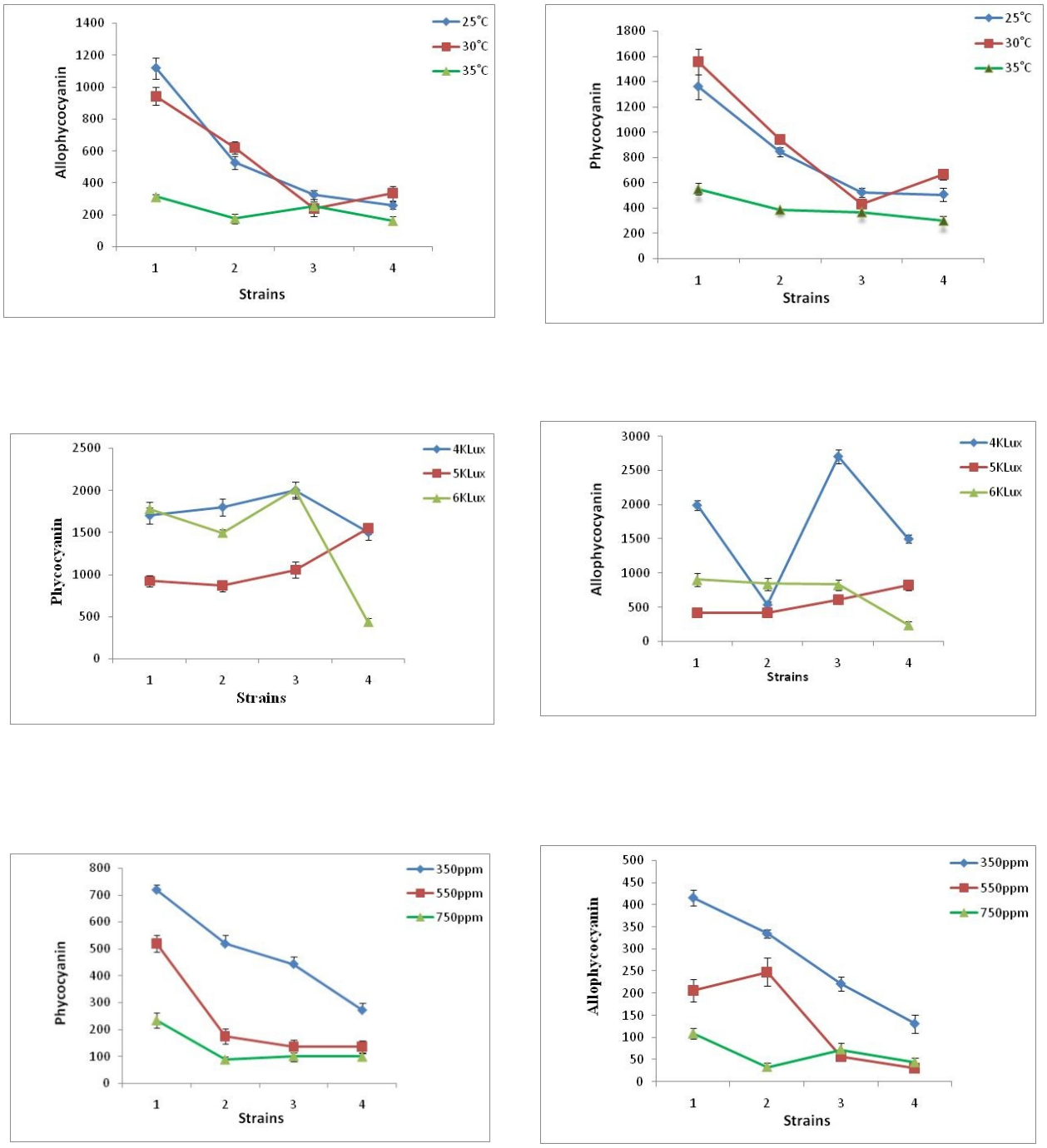


Figure 2: Comparative phycocyanin and allophycocyanin (µg/g dry weight) at different temperatures (25^{0}C, 30^{0}C, and 35^{0}C), light intensities (4KLux, 5KLux and 6KLux) and CO_2 concentrations (350ppm, 550ppm and 750ppm) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean±SD); 1-*Oscillatoria*, 2-*Lyngbya*, 3-*Anabaena* and 4-*Microchaete*.

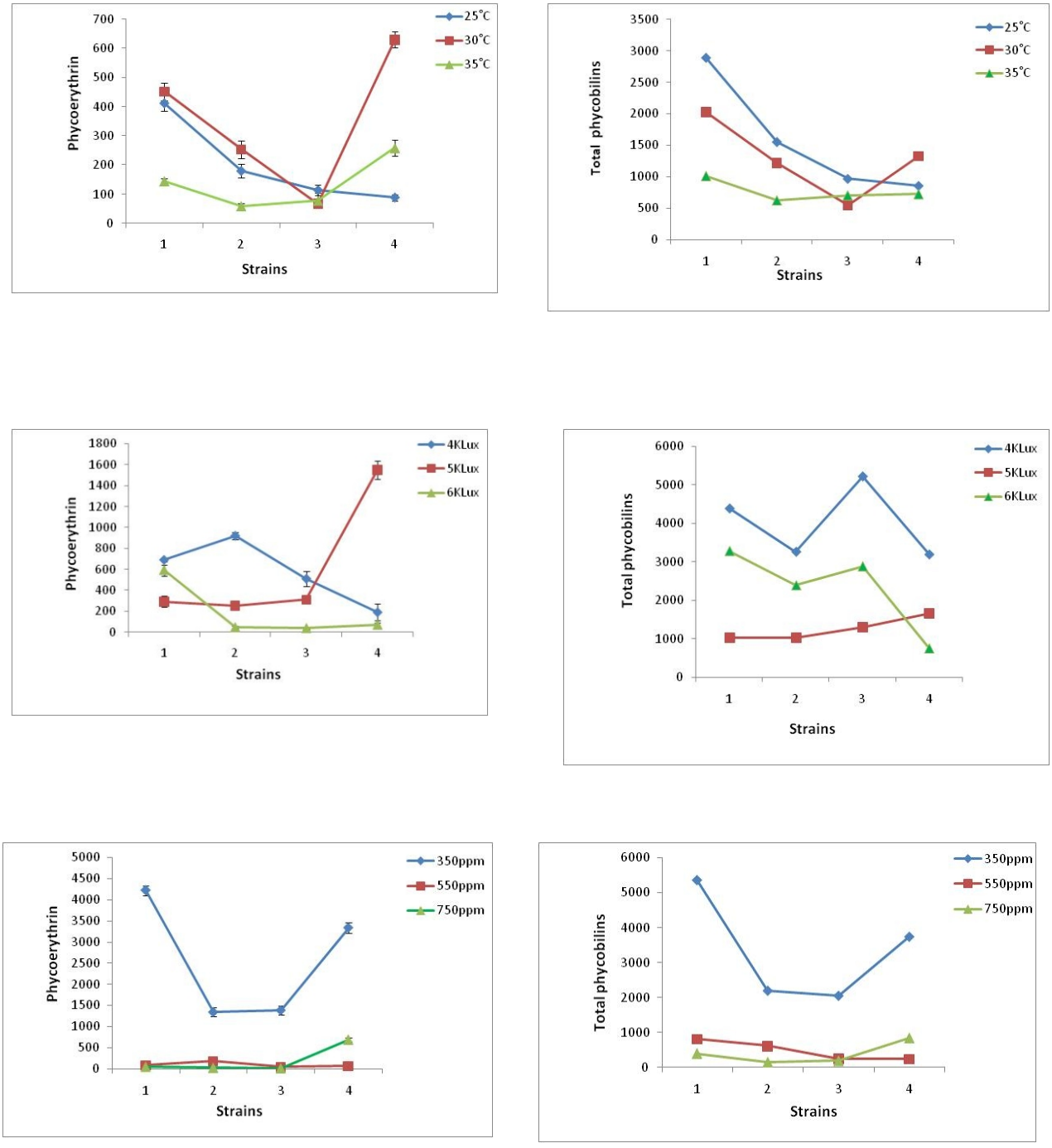


Figure 3. Comparative phycoerythrin and total phycobilins (µg/g dry weight) at different temperatures (25^{0}C, 30^{0}C, and 35^{0}C), light intensities (4KLux, 5KLux and 6KLux) and CO_2 concentrations (350ppm, 550ppm and 750ppm) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean ± SD); 1-*Oscillatoria*, 2-*Lyngbya*, 3-*Anabaena* and 4-*Microchaete*.

comparison to control grown cultures. In *Lyngbya* the highest allophycocyanin was recorded in glutamine supplemented medium. Inclusion of $ZnSO_4$ either alone or with glutamine decreased the allophycocyanin in *Lyngbya* as compared to control. Phycoerythrin enhanced with glutamine supplementation alone in *Oscillatoria* and *Lyngbya* in comparison to control grown cultures. $ZnSO_4$ alone depicted an inhibitory effect in *Oscillatoria*. Glutamine and $ZnSO_4$ taken together enhanced phycoerythrin content significantly in *Lyngbya* as compared to control. Highest phycoerythrin was recorded in glutamine supplemented medium followed by ZnSO4 supplemented medium. In general glutamine and $ZnSO_4$ together enhanced total phycobilins as compared to control in *Oscillatoria* whereas in *Lyngbya* significant enhancement in total phycobilins was observed with glutamine or with $ZnSO_4$ compared to control. These together did not influence total phycobilins in two genera. The amount of high levels of phycocyanin and carotenoids under specific optimised condition can have a biotechnological potential in the utilization of natural colours. These pigments represent 20 percent of the total dry weight in cyanobacterial genera (Borowitzka 1988, Walsh 1998). This indicates the possibility of using Zn and glutamine for scaling up the production of phycobilins in this culture as these pigments are increasingly being used in diagnostic and biomedical research including fluorescence immune assays (Romay *et al*. 2003). Studies have also indicated that phycobilins of cyanobacteria have antioxidant, anti-inflammatory and neuroprotective properties (Kumar *et al*. 2003 and Romay *et al*. 2003). Some strains of *Anabaena* and *Nostoc* are rich in phycoerythrin (Guerrero *et al*. 1990) and these can be used as pigments or colourants in food industry as well as for cosmetics.

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REFERENCES

Bryant DA 1982 Phycoerythrocyanin and phycoerythrin: properties and occurance in cyanobacteria. *J. Gen. Microbiol*. **128** 835-844.

Sidler WA 1994 Phycobilisome and phycobiliprotein structures. In: *The Molecular Biology of Cyanobacteria,* (ed.) Bryant DA, Kluwer Academic, Dordrecht, Netherlands. Pp. 139-216.

Bartley GE & Scolnick PA 1995 Plant carotenoids: Pigments for photoprotection, visual attraction and human health. *The plant cell* **7** 1027-1038.

Bennett A & Bogorad L 1973 Complimentary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol.* **58** 419–435.

Borowitzka M A 1988 Vitamins and fine chemicals from microalgae. In: *Micro-algal Biotechnology*. (Eds.) Borowitzka M A & Borowitzka L J Cambridge: University Press, pp.153-196.

Boussiba S & Richmond A 1979 Isolation and characterization of phycocyanin from blue green alga *Spirulina platensis*. *Arch. Microbiol*. **120** 155-159.

Clayton M N & King R J 1990 *Biology of Marine Plants*. Longman Cheshire, Melbourne.

Melbourne Cochran W G & Cox G M 1957 *Experimental Designs*. John Wiley & Sons , Inc., New York Pp. 127-131.

Guerrero M G, Vargas A M, Rivas J, Rodriguez H, Moreno J & Losada M 1990 Cultivation of nitrogen fixing micro-algae for the generation of products with commercial interest. In: *From Genes to Bioproducts*. (Ed). Organising committee of biotec. **90** 91-97. PPU DM. MURACIA.

Hertzberg S, Liaaen-Jensen S & Siegelman HW 1971 The carotenoids of blue-green algae. *Phytochem*. **10** 3121-3127.

Humm HJ & Wicks SK 1980 *Introduction and guide to the marine blue green algae*. John Willey and Sons Inc, New York, Pp. 121-123.

Jensen A 1978 Chlorophylls and carotenoids. In: *Phycological Methods: Physiological and Biochemical Methods* (eds Hellebust J A & Craige J S), Cambridge University Press, Cambridge, Pp. 59–70.

Kumar K, Lakshmanan A & Kannaiyan S 2003 Bioregulatory and therapeutic effects of blue green algae *Indian journal of Microbiology*, **43**, 9-16.

MacColl R & Guard-Friar D 1987 *Phycobiliproteins*. CRC Press, Boca Raton,FL.

McKinney G 1941 Absorption of light by chlorophyll solutions. *J Biol Chem* **140** 315-322.

Padgett M P & Krogmann DW 1987 Large scale preparation of pure phycobiliproteins *Photosynth. Res*. **11** 225-235.

Romay C, Gonzalez R, Ledon N, Remirez D, & Rimbau V 2003 c-Phycocyanin: A biliprotein with antioxidant, anti-inflammatory and neuroprotective effects, *Current Protein and Peptide Science*, **4** 207-216.

Stanier P, Henson J N, Moore G E & Copp A J 1995 Genetic basis of neural tube defects: the mouse gene *loop-tail* maps to a region of choromosome I syntenic with human 1q21-22, *Genomics* **26** 473–478.

Walsh K, Jones G J & Dunstan R H 1997 Effect of irradiance on fatty acid, carotenoid, total protein composition and growth of *Microcystis aeruginosa*. *Phytochemistry* (UK) **44** 817-824.

Yamaguchi T 1981 Strong deodrant. *Japanese Patent 79 JP-116131*.