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Impact of natural light on growth and biopigment profile of cyanobacteria *Spirulina platensis*

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Abstract

Cyanobacteria are economically important microorganisms and good source of natural pigments such as chlorophyll, carotenoids and phycobiliproteins. The present research work showed the optimum combination of photophase and scotophase of *Spirulina platensis* on biomass and chlorophyll-a, carotenoids, phycocyanin, allophycocyanin, and phycoerythrin contents. The study revealed that among all six light conditions tested, the cultures placed at west facing window, receiving natural day light at temperature 30°C showed extremely significant higher biomass (O.D. 3.46±0.17%) and biopigment accumulation Chlorophyll a 8.94±0.43%, Carotenoid 1.62±0.18%, phycocyanin 2.26±0.14%, allophycocyanin 2.66±0.18% and phycoerythrin 1.32±0.31% as compared to the standard (Full day natural light), which might be beneficial for large scale production of biopigment.

Key words

Carotenoids, Chlorophyll-a, Light condition, Phycobiliproteins, *Spirulina platensis*

Introduction

Spirulina platensis has been commercially used in several countries as health food, feed (Belay, 2002; Becker, 2003), bio-fertilizers (Vaishampayan *et al.*, 2001), antimicrobial agents (Kumar *et al.*, 2013), anti-cancer drugs, antioxidants (Estrada *et al.*, 2001) and biotechnology (Eriksen, 2008) because of its valuable constituents like phycobiliproteins, carotenoids, vitamins, polysaccharides, minerals and polyunsaturated fatty acids such as gamma-linolenic acid (Khan *et al.*, 2005; Mani *et al.*, 2005).

Phycobiliproteins are water soluble and highly fluorescent proteins, very stable at physiological pH. Phycobiliproteins are gaining importance as natural colorants over synthetic colour as they are nontoxic and non-carcinogenic (Chaneva *et al.*, 2007). Among the phycobiliproteins derived from *S. platensis*, the most abundant is phycocyanin, a brilliant blue colour pigment having great importance because of its various biological and pharmacological properties e.g. antioxidant, antiviral, anticancer (Liu *et al.*, 2000), neuro-protective (Romay *et al.*, 2003), antitumor (Li *et al.*, 2005), radical scavenging and anti inflammatory properties (Vadiraja and Madyastha, 2000). Carotenoids are structurally diverse lipid soluble pigment having many different biological functions, such as specific coloration, photoprotection, light harvesting and also serving as precursors for many hormones; therefore an important medicinal and biotechnological class of natural pigments (Pulz and Gross, 2004).

Many factors affect growth and biopigment accumulation of microalgae, including light (duration and intensity), nutrients availability, pH and temperature (Samuel *et al.*, 2010; Pandey *et al.*, 2011). Composition and content of phycobiliproteins in cyanobacteria are influenced by environmental factors (Chaneva *et al.*, 2007; Hemlata and Fatma, 2009; Simeunovic *et al.*, 2013). Light is an important factor defining optimal conditions for the culture because irradiance directly influences photosynthetic mechanism. A relatively low morning temperature with rapid increase in light intensity can induce photoinhibitory stress, and heating the cultures significantly reduces growth and biopigment accumulation. These factors affect the nutritional value of microalgae (Brown and Hohmann, 2002; Sayegh and Montagnes, 2011).

In view of the above, the present work was carried out to assess the influence of photophase (light) and scotophase (dark)

on biomass and biopigment contents in *S. platensis* to optimize the best light condition for improvement of biomass and biopigment accumulation.

Materials and Methods

Microorganism's culture condition and biopigment analysis : The experimental organism *S. platensis* was isolated from Jal mahal, Jaipur, Rajasthan (India) and cultivated under different temperatures and illuminated with white fluorescent lamp at light intensity of 2,000 lux. Experiments to evaluate the effect of different light condition were carried out in departmental laboratory. Conical flasks of 250 ml capacity were prepared containing 100 ml *S. platensis* culture with initial optical density 0.1. Cultures were shaken gently thrice a day to avoid clumping and enhanced the growth.

S. platensis was cultured under six different conditions and group 1 was considered as standard which received full day natural light at 30±2°C (FDNL). Group 2, 3 and 4 received 8:16, 16:8, 12:12 alternate light and dark (ALD) hrs respectively at a light intensity of 2,000 lux and 25±2°C. Group 5 received natural day light (NDL) at west facing window and temperature 30°C. Group 6 received constant light condition at a light intensity of 2,000 lux and 25±2°C.

Observations were carried out over a period of 30 days after initial readings. Growth was determined by optical density of cultures at 670 nm using Systronics UV-VIS spectrophotometer. The effect of these treatments on growth was analyzed biochemically for their biopigments by following the standard methods of Parson and Strickland (1965) for chlorophyll content, Bennet and Bogorad (1971) for phycobiliprotein content and Jenson (1978) for carotenoid content respectively. Three replicates used for each biochemical analysis.

Statistical analysis : Statistical evaluation of the results was made with SPSS 16.0 (SPSS Inc. Chicago, Illinois, USA). All values were expressed as mean±SE. Difference in mean of growth and biopigment accumulation of *S. platensis* were statistically analyzed by one-way ANOVA. Probability value $P \leq 0.05$ were regarded as statistically significant.

Results and Discussion

Light and temperature are the main factors influencing photosynthesesis, consequently affecting biomass and biopigment accumulation. The study revealed that after 30 days experimental groups showed significant difference in growth as compared to the standard, except 8:16ALD (p= 0.6188) and CLC (p=0.4954). Among all the six light conditions tested, culture placed at west facing window receiving natural day light (NDL) showed extremely statistically significant higher growth (O.D. 3.46±0.17, p=0.0001) followed by CLC (O.D. 3.43±0.27), 16:8 ALD (O.D. 2.32±0.25), 12:12 ALD (O.D. 2.10±0.13), 8:16 ALD (O.D. 0.93±0.12) as compared with to (O.D. 0.86±0.05) (Table-1). These observations were also supported by higher contents of phycobiliproteins and chlorophyll-a. However, carotenoid content showed different trend. It was observed that up to 10th day of experiment, growth under continuous illumination was greater than alternate light and dark condition. This was due to the incidence of adequate light energy under continuous light increased during cell metabolism process.

In FDNL (Group-1), net biomass was found to be lower because of high temperature and high light intensity that resulted in reduction of biomass because water loss could have changed in the osmotic pressure in culture medium leading to cells damage (Kumar *et al.*, 2013).

Chlorophyll-a content correlated well with biomass of *S. platensis* (Pearson correlation coefficient 'r' =0.871). Chlorophyll-a content under all light condition, as compared to the standard, was found to be statistically significant. Maximum Chl-a was found in NDL (8.94±0.43%) followed by 16:8 ALD (6.40±0.24%), whereas minimum chlorophyll-*a* was observed in 8:16 ALD (3.12±0.16%) in (Table-1). Culture receiving full day natural light (Group-1), Chl-a content found to lowest (0.76±0.08%). After 20 day onwards, in continuous light, growth and chlorophyll content reduced due to photooxidation as excess light cannot be absorbed by the photosynthetic apparatus (Kumar *et al.*, 2011).

Carotenoid content was highly correlated with growth of *S. platensis* as Pearson correlation coefficient 'r' was found to be 0.90. Carotenoid content was found significantly different in NDL (1.62±0.18%) followed by CLC (1.39±0.21%), (1.24±0.12%) 12:12ALD (1.23±0.16%) in 16:8ALD, except for 8:16ALD which was not significantly difference (0.72±0.05%) as compared to FDNL which was found to have lowest carotenoid content (0.22% Table 1).

In FDNL, carotenoid content increased rapidly up to 20th day to show the adaptive mechanism by *S.platensis* for photoprotection against photo-oxidative damage of cell (Simeunovic *et al.*, 2013).

Phycocyanin was found to be highly correlated with growth as Pearson correlation coefficient (r =0.784), was within the range of 1-0.75. Chl-a (r=0.810), carotenoid (r=0.817). Phycocyanin content showed significant difference in all groups as compared with standard. Highest amount of phycocyanin was found in NDL (2.26±0.14%) followed by 16:8ALD (2.22±0.27%), CLC (1.91±0.03%), 12:12ALD (1.84±0.08%), 8:16ALD (0.96±0.10%) as compared with Group-1(Table 1).

Allophycocyanin showed positive correlation with biomass and Pearson correlation coefficient 'r' found to be 0.833. In groups APC content extremely statistically significant difference were shown except 8:16ALD (p=0.1129) and 12:12ALD (p=0.0570). Highest amount of allophycocyanin was

Table 1 : Effect of different light condition on growth and biochemical profile (percent) of *S.platensis* after 30 days of treatment

Treatment	Growth	Chlorophyll a	Carotenoid	Phycocyanin	Allophycocyanin	Phycoerythrin
STD	0.86±0.05	0.76±0.08	0.22±0.02	0.54±0.07	0.78±0.09	0.52±0.04
8:16ALC	0.93ns±0.12	3.12**±0.16	0.72ns±0.05	0.96*±0.10	1.28ns±0.23	0.89*±0.12
16:8ALC	2.32**±0.25	6.40**±0.24	1.23**±0.16	2.22**±0.27	2.03**±0.16	1.05*±0.14
12:12ALC	2.10**±0.13	5.34**±0.13	1.24**±0.12	1.84**±0.08	1.41*±0.22	0.90ns±0.21
NDL	3.46**±0.17	8.94**±0.43	1.62**±0.18	2.26**±0.14	2.66**±0.18	1.32*±0.31
CLC	3.43ns±0.27	6.00**±0.13	1.39**±0.21	1.9**±0.03	2.31**±0.12	1.06**±0.06

Value are mean of three replicates ±SE; ns= non Significant ($p \geq 0.05$); *=Significant ($p \leq 0.05$); **= Highly Significant ($P \leq 0.0005$)

observed in NDL (2.66±0.18%) followed by CLC (2.31±0.12%), 16:8ALD (2.03±0.16%), 12:12ALD (1.41±0.22%), 8:16ALD (1.28±0.23%) as compared with standard (Table 1).

Phycoerythrin showed moderate correlation with allophycocyanin (r = 0.71). PE value was content found to be statistically significant, except for 12:12ALD. Highest amount of PE was observed in NDL (1.32±0.31%) followed by CLC (1.06±0.06%) 16:8ALD (1.05±0.41%), 12:12ALD (0.90±0.21%), 8:16ALD (0.89±0.12%) (Table 1). The increase or decrease in the level of phycobiliproteins depends on cellular and metabolic activities of the organism which was affected by duration of light and temperature. High temperature showed decrease in PBPs' content in *S. platensis* due to photo inhibition and photo-oxidation (Seyfabadi *et al.*, 2011). Culture receiving constant light, PBPs content did not show linear progressive growth as compared to alternate light and dark culture condition at 25±2°C. The results of the present study revealed that in 16:8 alternate light and dark exponential increase in PBP content was found as compared to constant light at 25±2°C. In general, high PBPs content was exhibited in rapidly growing microalgal cells (Sayegh and Montagnes, 2011). So the rapidly growing culture of natural day light (Group-5) placed at west facing window, showed significant and higher amount of PC, APC and PE.

On the basis of observation it is concluded that biopigment showed high correlation with biomass of *S. platensis.* These results suggest that maximum biopigment content shown by the culture receiving natural day light at west facing window at 30±2°C. Under laboratory conditions culture receiving 16:8 hour light and dark showed high phycobilliproteins accumulation at 25±2°C and 2,000 lux light intensity.

The present study confers the result of Kumar *et al.,* 2011 that Light/dark cycle was more helpful for growth than other regimes and also save the consumption of light energy and increase efficiency.

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