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Effect of Carbon Content, Salinity and pH on *Spirulina platensis* for Phycocyanin, Allophycocyanin and Phycoerythrin Accumulation

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Abstract

The cyanobacterium *Spirulina platensis* is an attractive source of the biopigment, which is used as a natural colour in food, cosmetic, pharmaceutical products and have tremendous applications in nutraceuticals, therapeutics and biotechnological research. The present study examines the possibility of increasing the content of Phycocyanin, Allophycocyanin, Phycoerythrin and Carotenoids under stress conditions including different pH, salinity and carbon content in *S. platensis* isolated from Jalmahal, Jaipur (Rajasthan). The production of Phycocyanin, Allophycocyanin and Phycoerythrin were enhanced with 0.4 M NaCl, pH 7 and Carbon deficiency as compared to standard.

Keywords: *Spirulina platensis*; Phycobilliproteins; Chlorophyll-a; Carotenoids; Abiotic stress

Introduction

Spirulina platensis has been commercially used in several countries as health foods [1,2], feed [3], bio-fertilizers [4] and applications in biotechnology [5] because of its valuable constituents such as proteins, vitamins, minerals, carbohydrates, lipids and polyunsaturated fatty acids. They have anti-cancer properties [6] and immune promoting effects [7]. *S. platensis* is an attractive source of various bioactive substances such as sterols function as antimicrobial agents [8,9], polysulfated polysaccharides as antiviral agents [10], phycobilliproteins and carotenoids as antioxidants [11], mycosporine-like amino acids (MAAs) and scytonemin as photoprotectants [12], polyunsaturated fatty acid (PUFA) as serum lipids levels reduction and HDL-cholesterol increasing [13], Gamma-linolenic acid (GLA) as rheumatoid arthritis [14], eczema [15], diabetes, multiple trauma, and premenstrual syndrome [16].

Phycobilliproteins are water soluble and highly fluorescent proteins, very stable at physiological pH [17]. Phycobilliproteins are gaining importance as natural colorants over synthetic colour, as they are nontoxic and non-carcinogenic [18]. Among the phycobiliproteins derived from *S. platensis*, the most abundant is phycocyanin (PC), a brilliant blue colour pigment have greater importance because of its various biological and pharmacological properties e.g. antioxidant [19], antiviral [20], anti-cancer [21], neuro-protective [22], hepatoprotective [23], antitumor [24], radical scavenging [25], radioprotection [26] and anti-inflammatory properties [27]. Carotenoids are structurally diverse lipid soluble pigment have many different biological functions, such as specific coloration, photoprotection, light harvesting and also serve as precursors for many hormones therefore an important medicinal and biotechnological class of natural pigments [28].

In addition, phycobiliproteins are widely used in immunological assays, due to high fluorescence, good storage stability at temperatures between 4-10°C, high molar absorbance coefficient, high photo-stability, isoelectric point (IP) close to 4.65, making them easily linkable to antibodies without changing its spectral properties [29].

Environmental stresses affect growth and biopigment accumulation of microalgae, including nutrients availability, high pH, light, salinity and temperature [30]. The culture conditions can influence the growth phases of *Spirulina platensis*, causing changes in its composition and proportion of phycobiliproteins [31]. Studies indicated that the quantities of phenolic compound increased by altering the culture conditions and enhance the antioxidant potential of *S. platensis* biomass exploit as a nutritional supplement [32].

Salt stress inhibits plant growth and productivity which are often associated with the decreased photosynthesis [33]. A number of studies have been performed to study the effect of salt stress. Ionic stress due to 0.5 M NaCl inactivated photosynthetic machinery in *Synechococcus* sp [34]. Biswal et al. [35] have revealed that salt stress (0.5 M NaCl) caused inhibition in photosynthetic electron transport in *Brassica junica*. The decrease in PS II activity in *Chlamidomaonas reinhardtii* has been found to be associated with state 2 transitions [36]. In *Triticum aestivum* light enhanced the inhibitory effect of salt stress on PS II efficiency [37].

The pH plays important role in the metabolic activities of microalgae. It strongly affects biomass production, chemicals dissociation and cell physiology. Therefore, the effect of different pH levels on the growth of microalgae was continuously evaluated under different environmental condition [38,39].

The purpose of the present work was to assess the influence of stress conditions including pH, salinity and carbon content on the biomass and biopigment accumulation in *S. platensis* to optimize the best culture condition for improvement of Phycocyanin (PC), Allophycocyanin (APC), Phycoerythrin (PE), Chlorophyll-a (Chl-a) and Carotenoids (Cart).

Materials and Methods

Microorganism and culture condition

The experimental organism *S. platensis* was isolated from Jal Mahal, Jaipur, Rajasthan (India) and cultivated in Zarrouk's medium

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[40]. Experiments to evaluate the effect of different stress conditions were carried out in departmental laboratory. Conical flasks of 100 ml capacity were prepared containing 50 ml *S. platensis* culture with initial optical density 0.1 for all treatment groups. The cultures placed at west facing window receiving natural day light at temperature 30 ± 2°C and shaken gently thrice a day to avoid clumping and enhance the growth.

Experimental design

For this study Zarrouk's medium modified with carbon source content, salinity level and pH. All treatment groups are summarized in Table 1.

Analytical methods

Observations were carried out weekly over a period of 30 days after initial readings. Culture growth was determined by optical density at 670 nm by using Systronics UV/ VIS spectrophotometer. The effect of these treatments on growth analyzed biochemically for their biopigments by using standard methods suggested by Parson and Strickland [41] for chlorophyll, Bennet and Bogorad [42] for phycobiliproteins, Jenson [43] for carotenoids content of the cultures was measured quantitatively.

Growth measurements

Measurement of optical density (O.D.) is particularly suitable for determination of growth of *S. platensis*. The basic advantage of turbidity technique in growth rate measurements is the possibility of taking repeated readings on increase in turbidity of the same batch of the suspension of cells.

Chlorophyll estimation

5 ml of homogenized cyanobacterial suspension was taken and subjected to centrifugation for 10 minute at 4000 rpm. The chlorophyll-a (Chl-a) was extracted from pellet by using 5 ml 90% acetone. Place the tube in dark for 24 hour. After extraction period centrifuge the sample for 15 min at 5000 rpm and collect the supernatant. Read the absorbance at 630 nm (A630), 645 nm (A645), and 665 nm (A665) against 90% acetone as blank by using Systronics UV/ VIS spectrophotometer and the concentration of Chl-a was calculated using the formula:

C = 11.6 A665–1.31 A645–0.14 A630

The concentration of Chl-a in a given volume of culture can be determined by formula:

$$\text{Chl-a ((mg/l))} = \frac{C \times Ve}{Vc}$$

Group	Treatment
G1.	Standard ($NaHCO_3$ -18.0 g/l, 0.017 M salinity, pH 9)
G2.	–100% Carbon deficiency ($NaHCO_3$ - 0.0 g/l)
G3.	–75% Carbon deficiency ($NaHCO_3$ - 4.5 g/l)
G4.	–50% Carbon deficiency ($NaHCO_3$ - 9.0 g/l)
G5.	0.2 M Salinity
G6.	0.4 M Salinity
G7.	0.6 M Salinity
G8.	0.8 M Salinity
G9.	pH 6
G10.	pH 7
G11.	pH 10
G12.	pH 11

Three replicate were made for each treatment group.

Table 1: Experiments to evaluate the effect of different stress conditions.

C=Value obtained from above equation

Ve=Volume of extract (ml)

Vc=Volume of culture (litres)

Phycobiliproteins estimation

5 ml of cyanobacterial cell suspension was taken and centrifuged to obtain the pellet for 10 minute at 4000 rpm. Wash the pellet with distilled water and Phycobiliproteins were extracted completely from pellet with 5 ml of phosphate buffer (0.05 M, pH 6.7) by three times repeated freezing and thawing. Centrifuged the sample for 15 min at 10,000 rpm and collect the supernatant. The absorbance was read at 562 nm (A562), 615 nm (A615), and 652 nm (A652) against phosphate buffer as blank by using Systronics UV/ VIS spectrophotometer and concentration of phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE) were calculated by using the formula:

$$\text{PC} = \frac{A615 - 0.474(A652)}{5.34}$$

$$\text{APC} = \frac{A652 - 0.208(A615)}{5.09}$$

$$\text{PE} = \frac{A562 - 2.41(PC) - 0.849(APC)}{9.62}$$

The concentration of phycobiliprotein in a total volume of culture can be determined as follows:

$$\text{Phycobiliprotein (mg/ml)} = \frac{C \times Ve}{Vc}$$

C=Value of PC, APC and PE obtained from above equations

Ve=Volume of extract (ml)

Vc=Volume of culture (ml)

Carotenoids estimation

5 ml of homogenized cyanobacterial suspension was taken and subjected to centrifugation for 10 minute at 4000 rpm. Wash the pellet 2-3 times with distilled water to remove traces of adhering salts. Harvested biomass is broken down by the pestle and mortar with 5 ml, 90% acetone and centrifuged the sample at 15 min at 5000 rpm. Collect the supernatant and read the absorbance at 450 nm (A450) by using Systronics UV/ VIS spectrophotometer. Carotenoids (Cart) concentration calculated by using formula:

$$\text{C} = \frac{A450 \times V \times f \times 10}{2500}$$

C=Total amount of Cart (mg/ml)

V=Volume of extract (ml)

f=Dilution factor

Statistical analysis

Statistical evaluation of the results was made with SPSS 16.0 (SPSS Inc. Chicago, Illinois, USA). All values were expressed as mean ± SEM. The differences in the mean of growth and biopigment accumulation of *S. platensis* were statistically analysed by one-way ANOVA followed by Post hoc Dunnett t test. In view of the exploratory nature of the study, probability values $P \leq 0.05$ were regarded as statistically significant.

Results and Discussion

The effect of different stress conditions on the growth of *S. plantensis*

expressed in terms of optical density at 670 nm. Growth and biopigment analysis were evaluated weekly during 30 days of cultivation. Post hoc tests included to determine the statistical significance between all groups. It was observed that the biomass of *S. platensis* was inhibited by all abiotic factors such as pH, salinity, carbon deficiency applied.

Among all the condition tested, G-2 (-100% carbon deficiency) had shown very significant higher content of chlorophyll-a (Chl-a) i.e. 10.58 ± 0.40 followed by G-10 (pH 7) i.e. 9.05 ± 0.33, 8.80 ± 0.63 in G-5 (0.2 M salinity) as compared to standard (8.13 ± 1.26), whereas minimum Chl-a content observed in G-12 (pH 11) i.e. 3.68 ± 0.20 (Figure 1,2a-2c). The decreased content of Chl-a mainly due to decreasing the free carbon dioxide concentration in the medium at high pH (>10) because in this pH the carbonate form is predominant and the bicarbonate form is the one utilized by *S. platensis* [44].

Carotenoids was found to be higher content in G-10 (pH 7) i.e. 6.59 ± 0.60 followed by G-9 (pH 6) i.e. 6.19 ± 0.17 as compared to standard (6.12 ± 0.12). G-8 (0.8 M salinity) had shown minimum content of carotenoids i.e. 1.55 ± 0.16 (Figures 1,2a-2c).

The study revealed that after 30 days of experimental groups

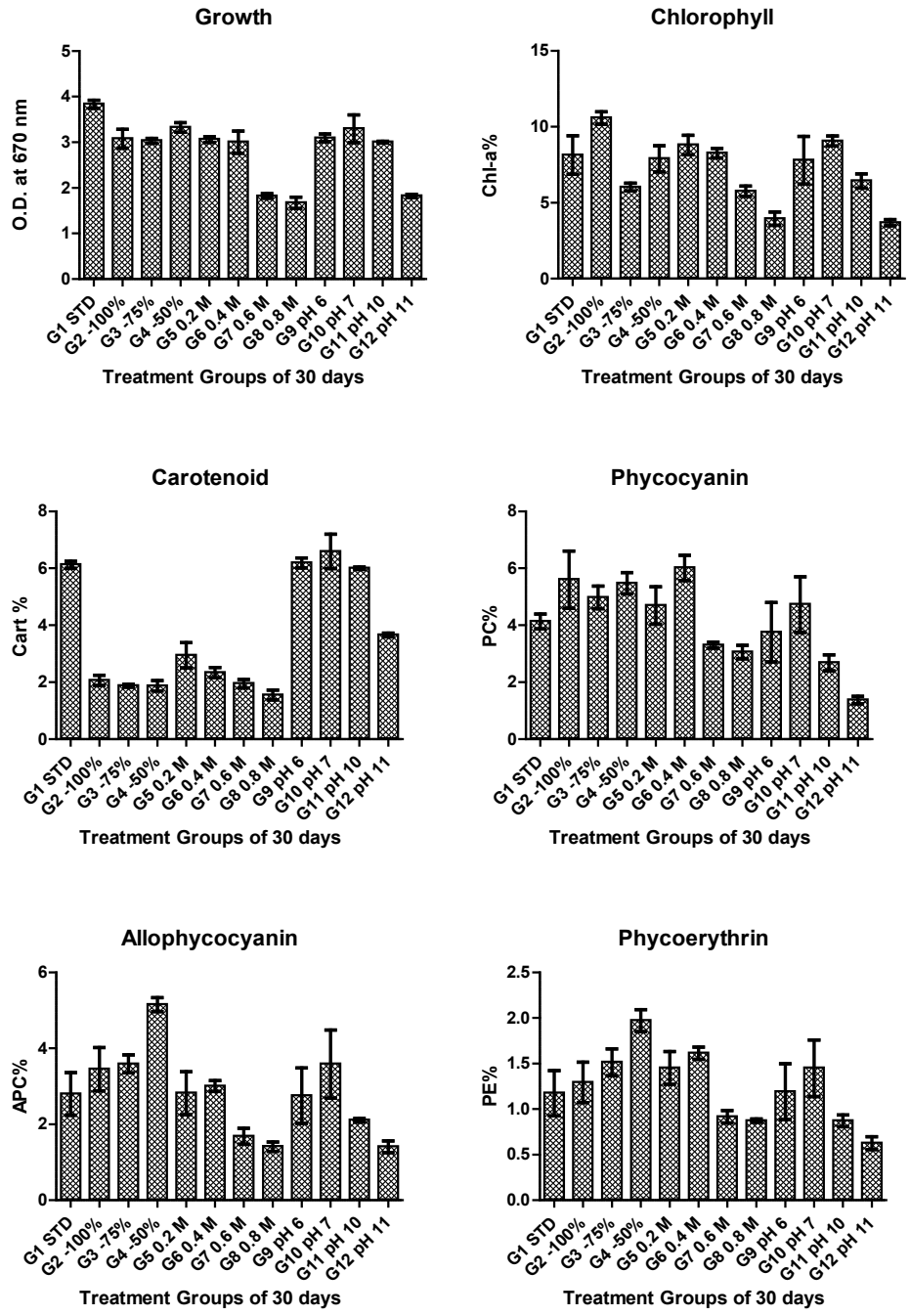

Figure 1: Effect of Carbon content (G2 to G4), Salinity (G5 to G8) and pH (G9 to 12) on Growth, Chlophyll-a, Carotenoids, Phycocyanin, Allophycocyanin and Phycoerythrin content of *S. platensis*. Value are means±SEM (n=3).

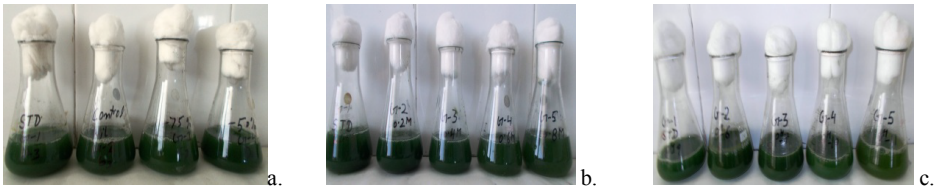

Figure 2: Effect of Carbon content (a), Salinity (b) and pH (c) on Growth, Chlophyll-a, Carotenoids, Phycocyanin, Allophycocyanin and Phycoerythrin content of *S. platensis*.

had shown significant difference in content of phycocyanin (PC) as compared to standard. G-6 (0.4 M salinity) had shown very significant higher content of phycocyanin i.e. 6.01 ± 0.44 followed by G-2 (-100% carbon deficiency) i.e. 3.60 ± 1.00, 4.72 ± 0.97 in G-10 (pH 7) as compared to standard G-1(pH 9) i.e. 4.13 ± 0.26. The minimum amount of PC was found in G-12 (pH 11) i.e. 1.37 ± 0.13 as compared to standard (Figure 1,2a-2c).

The G-4 (-50% carbon deficiency) had shown very significant higher amount of allophycocyanin (APC) i.e. 5.15 ± 0.18 followed by G-10 (pH 7) i.e. 3.58 ± 0.89, 3.00 ± 0.14 in G-6 (0.4 M salinity) as compared to standard (2.79 ± 0.56). G-12 (pH 11) had shown minimum content of APC i.e. 1.40 ± 0.15 (Figures 1,2a-2c).

The value of phycoerythrin (PE) found to be significant higher amount in G-4 (-50% carbon deficiency) i.e. 1.97 ± 0.12 followed by G-6 (0.4 salinity) i.e. 1.61 ± 0.06, 1.44 ± 0.30 in G-10 (pH 7) as compared to standard. The lowest content of PE was found in G-12 (pH 11) i.e. 0.62 ± 0.07 as compared to standard (1.71 ± 0.24) (Figures 1, 2a-2c).

Environmental stress is the factor which affects mass cultivation of *S. plantensis* due oxidative damage at the cellular level. Oxidative stress is caused by an imbalance between the production of active oxygen and the ability to detoxify the peroxides and free radicals [45]. When different pathways are uncoupled, high energy electrons are transferred to molecular oxygen to form ROS [46]. Disturbances in this normal redox state can cause highly toxic effects through the production of reactive oxygen species (ROS) that damage all components of the cell including proteins, lipids and DNA. To overcome the challenges of oxidative damage *S. plantensis* developed enzymatic antioxidants and non-enzymatic antioxidants [47].

It was observed that an increase of NaCl concentration caused reduction of growth and total inhibition of chlorophyll-a (Chl-a) biosynthesis of *S. platensis*. This is due to an energy level decline caused by pumping out the entering sodium ions [48]. The increase in the salt concentration caused significant inhibition of electron transport chain and photosystem (PS-II) catalysed electron transport due to damage of reaction centre of PS-II and alterations in water oxidation complex [28].

pH is one of the environmental factors which affect the physiological growth, metabolic activities and biomass production of *S. platensis*. The results demonstrate that *S. platensis* can adapt to variable pH conditions as suggested earlier [38,39].

The obtained results showed that any changes in Carbon content led to a significant effect on growth and phycobiliproteins accumulation since photorespiration which protects the photosynthetic membrane against light induced damage at times when carbon assimilation is limited [45]. Photosynthetic rate of *S. platensis* was higher in the medium containing higher HCO^{3-}. The rate of CO_2 fixation in cyanobacteria depends upon the accumulation of inorganic carbon sources [49].

Poza-Carrion et al. [50] revealed that increasing pH (7-9) significantly increased the phycobiliproteins content in *Nostoc* sp. Abd El-Baky [51] reported that increasing in salinity levels in nutrient medium led to significant increase in phycocyanin and other soluble proteins content in *Spirulina maxima*.

Conclusion

In this paper we have demonstrated that stress conditions influence the production of biomass, phycobiliproteins, chlorophyll-a, carotenoids content of *S. platensis*. The phycobiliproteins contents of *S. platensis* were increased at 0.4 M NaCl as well as pH 7. This can be used in large scale production of phycobiliproteins, which can solve the problem of availability of protein sources in a number of commercial applications.

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