

Optimization of production of phycobiliproteins from *Spirulina maxima* and *Spirulina platensis* under laboratory conditions

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ABSTRACT

Cyanobacteria, especially *Spirulina* sp., are considered as valuable tool for obtaining phycobiliproteins, which has numerous applications. The present study was conducted to optimize the production of phycobiliproteins from two strains of *Spirulina*, i.e. *S. maxima* and *S. platensis*, grown in CFTRI medium. The optimization was done by changing the light colour, nitrate source and pH of the medium. The results showed that monochromatic light, in red zone, nitrate source and sodium and potassium nitrate as well as pH 10.0 enhanced the production of three major components of phycobiliproteins, phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC), as well as chlorophyll a concentration and total cellular proteins, after 30 days of growth. Further, *S. platensis* proved to be better strain as compared to *S. maxima* for production of phycobiliproteins in CFTRI medium.

KEY WORDS; *Spirulina platensis*, *Spirulina maxima*, phycobiliproteins, phycocyanin, phycoerythrin, allophycocyanin

I. INTRODUCTION

Cyanobacteria (Blue green algae) are gaining scientific and industrial attention due to its multiple usage and simplicity of cultivation. These usage include, though not limited to sequestration of CO₂, biodiesel production, and an array of useful biological compounds, including pigments, proteins, toxins, and many more (Garlapati *et al.*, 2019). Among several value added products of cyanobacteria, its major light harvesting pigments i.e., chlorophyll-*a*, carotenoids and phycobiliproteins, are gaining higher response due to their colour and biological activities, apart from their light harvesting capacities (Singh *et al.*, 2009).

Phycobiliproteins, water soluble protein pigments, are known to possess numerous biological activities, i.e., antioxidant, anti-inflammatory, anti-viral, anti-cancer, neuro- and hepato-protective properties (Saini *et al.*, 2018). Further, as a natural pigment, they are useful as well as well utilized in a number of applications in foods and cosmetics, as fluorescent probes in various biotechnological and diagnostic applications (Rodríguez-Sánchez *et al.*, 2012; Jaiswal *et al.*, 2018). Natural colorants such as phycobiliproteins are gaining importance over synthetic ones, due to their nontoxic and non-carcinogenic properties (Fatma, 2009, Mandal *et al.*, 2020).

Among various cyanobacteria, *Spirulina platensis* has been shown to have higher capability to harvest light energy because of higher ratio between C- phycocyanin (C-PC) and Allophycocyanin (APC) (Ajayan *et al.*, 2012). Cyanobacteria also have been reported to synthesize phycobiliproteins, up to half of its total soluble protein (Muramatsu and Hihara, 2012).

Cyanobacteria are known to well adapt to the environmental stress signals, such as changes in nutrients, light intensity and wavelength and other physiological stress factors such as temperature and pH (Fatma, 2009).

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Further, environmental and physicochemical signals have been reported to have a significant role in triggering the synthesis of many value added products, including phycobiliproteins (Singh *et al.*, 2012). With these factors in mind, the present study is aimed to optimize the culture conditions with the aim to maximize the pigment productions from two strains of *Spirulina* under laboratory conditions.

II. MATERIALS AND METHODS

Organisms and culture conditions

The axenic culture of *Spirulina platensis* was obtained from Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi, India. *Spirulina maxima* was kindly provided by Dr. S. Singh, Centre of Advanced Studies in Botany, Banaras Hindu University, Varanasi, India. Both the cultures were maintained in CFTRI medium (Salunke *et al.*, 2016) as basal medium, and by routinely transferring (at interval of 6 d), the exponential phase cultures to 250 ml sterile medium. The cultures were grown photo-autotrophically in an air conditioned culture room maintained at $25\pm 1^\circ\text{C}$ and illuminated with cool day fluorescent lights of photon flux density $35\mu\text{Em}^{-1}\text{s}^{-2}$ on the surface of vessels for 16 h a day, with periodic shaking.

Experiments with variable wavelengths were performed with different monochromatic lights of visible range i.e., blue, green, yellow, red and pink on the cultures, as compared with that of control (white light). The culture flasks were wrapped in coloured cellophane papers to provide lights of different wavelength. The control culture flask was left uncovered. Other culture conditions remain the same. For pH variation, the *Spirulina* cultures were grown under different pH 8.0-12.0. The pH of the medium was adjusted before the commencement of the experiment by using 1N HCl and 1N NaOH. For effects of different nitrogen sources, the medium composition, in which the nitrogen source was sodium nitrate, was replaced by different nitrogen sources in same concentration, i.e., glycine, sodium nitrite, ammonium sulphate, urea, potassium nitrate and control without any nitrogen source.

Estimation of Chlorophyll a content

Growth of cyanobacterial culture was estimated by measuring the concentration of chlorophyll *a* and protein at a regular interval (72 h). The change in chlorophyll *a* content of cells was estimated following the method of Thingujam *et al.* (2016). Briefly, a known volume (3 ml) of culture was centrifuged at 3000g for 10 min and the supernatant was discarded. The pellet was washed with double distilled water and re-suspended in same amount (3 ml) of methanol followed by vigorous shaking by Cyclomixer. The sample was incubated at 60°C for 15 minutes and the optical density of the supernatant after centrifugation ($3000\times\text{g}$, 5 min) was read against methanol blank at 665 nm using Systronics 105 spectrophotometer. The chlorophyll *a* content was calculated using following coefficient:

$$\text{Chlorophyll } a \text{ in } \mu\text{g ml}^{-1} = A_{665} \times 13.42$$

Estimation of total protein content Total cellular protein content was estimated following the method of Lowry *et al.*, (1951) as modified by Nath *et al.* (2017). The amount of total cellular protein ($\mu\text{g ml}^{-1}$) was calculated by using standard curve prepared from Bovine serum albumin.

Estimation of phycobiliproteins

Estimation of Phycocyanin (PC), Phycoerythrin (PE), Allophycocyanin (APC) was done by the method of Bennett and Bogoard (1973). For this, a known volume (5 ml) of culture was centrifuged ($3000\times\text{g}$, 10 min), the supernatant was discarded and to the pellet, was added 5 ml of buffered saline (0.01M phosphate buffer, and 0.15 M NaCl). The pellet was cyclomixed vigorously and kept overnight in dark at room temperature for complete extraction. The tubes were centrifuged ($3000\times\text{g}$, 5 min), and the optical density of extracted pigments in the supernatant was read at 620 nm, 550 nm, and 650 nm, for PC, PE and APC respectively, against buffered saline as blank.

Relative concentrations were found by using the formula (Johnson *et al.*, 2014):

$$PC = OD_{620} - 0.474(OD_{650}) \div 5.34$$

$$PE = OD_{530} - 2.41(PC) - 0.849 (APC) \div 9.62$$

$$APC = OD_{650} - 0.208(OD_{620}) \div 5.09$$

III. RESULTS

In order to maximize the production of phycobiliproteins in CFTRI medium, various physiological parameters were changed. When the light was changed from white light to a monochromatic light, the growth of both of the *Spirulina* strains affected variably. Up to a 30 day time, while chlorophyll *a* concentration reached maximum with control (17.92 $\mu\text{g ml}^{-1}$), followed by light of pink colour only (16.64 $\mu\text{g ml}^{-1}$) for *S. maxima*. For *S. platensis*, chlorophyll *a* concentration reached maximum with control (18.97 $\mu\text{g ml}^{-1}$), followed by light of pink colour only (17.29 $\mu\text{g ml}^{-1}$). The total protein content for *S. maxima* after 30 days was found to be maximum (346.24 $\mu\text{g ml}^{-1}$) when pink light was provided, followed by yellow light (340.16 $\mu\text{g ml}^{-1}$). For *S. platensis*, the total protein content for *S. maxima* after 30 days was found to be maximum (352.64 $\mu\text{g ml}^{-1}$) when pink light was provided, followed by yellow light (340.20 $\mu\text{g ml}^{-1}$). Blue coloured light failed to promote growth of both the cultures, while green light was also less responding. Fig 1 shows the relative abundance of three cyanobacterial pigments, when the cultures were grown in white light (control), and monochromatic lights of different colours. The relative abundance of PC for *S. maxima* was found with pink coloured light (0.10), followed by white light. Similar data was achieved with *S. platensis*, with comparable relative abundances. Relative abundance of PE was higher in pink light (0.26), followed by yellow light (0.23) for *S. maxima*, while for *S. platensis*, PE abundance was higher in pink light (0.25) followed by white light (control, 0.23). The APC abundance was higher with pink and red light (0.16) followed by yellow light (0.14) for *S. maxima*. Experiments with *S. platensis* followed the similar trends as with *S. maxima*.

When nitrogen sources were varied and compared with that of conditions of no nitrogen source added to the CFTRI medium, the growth in terms of chlorophyll *a* concentration of *S. maxima* showed higher chlorophyll *a* concentration (11.35 $\mu\text{g ml}^{-1}$) when potassium nitrate was provided as a nitrogen source. The cultures supplied with glycine or were without nitrogen failed to grow beyond day 15. Similar observations were recorded with *S. platensis* with highest chlorophyll concentration (11.58 $\mu\text{g ml}^{-1}$) with potassium nitrate. The amount of protein was also higher (332.48 $\mu\text{g ml}^{-1}$) with potassium nitrate for *S. maxima*, and 335.04 $\mu\text{g ml}^{-1}$ for *S. platensis*. Fig 2 presents the relative abundance of three cyanobacterial pigments, when the cultures were grown different nitrogen sources. Potassium nitrate proved to be better nitrogen source for production of PC for *S. maxima*, while potassium nitrate and sodium nitrate showed equivalent effect on PC abundance in *S. platensis*. Similar trends were recorded with relative abundances of PE in the two cyanobacterial strains. The relative abundance of PE was also higher (0.078) with potassium nitrate, followed by sodium nitrate (0.070) for *S. maxima*. For *S. platensis*, the relative abundance of PE was equal (0.086) for both the nitrogen sources. The relative abundance of APC, however, presented a different story. The APC concentration was higher with sodium nitrate in both the strains, 0.071 for *S. maxima*, and 0.076 for *S. platensis*.

When the pH of the CFTRI medium was varied, the growth in terms of chlorophyll *a* concentration of both the strains were higher with pH 10.0, 12.77 and 13.23 $\mu\text{g ml}^{-1}$ for *S. maxima* and *S. platensis* respectively. The pH 10.0 was also found optimum for protein concentrations, which showed 295.68 and 314.24 $\mu\text{g ml}^{-1}$ for *S. maxima* and *S. platensis* respectively. Fig 3 presents the relative abundance of three cyanobacterial pigments, when the cultures were grown with different pHs. In all the cases, pH 10.0 was proved to be the best for relative abundances of PC, PE and APC, followed by pH 11.0, both for *S. maxima* and *S. platensis*. *S. platensis* produced higher amounts of PC, PE and APC as compared to *S. maxima*.

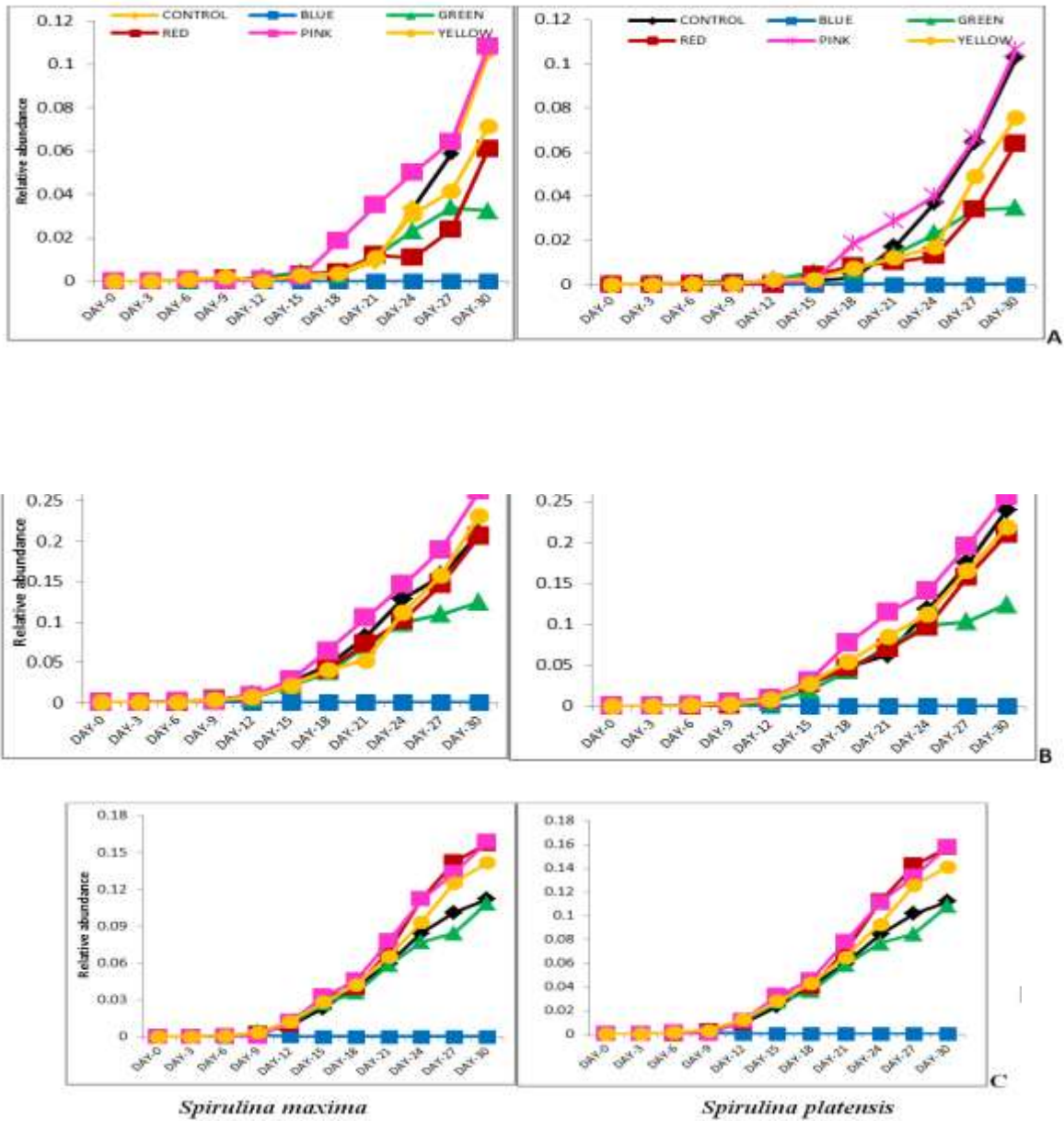
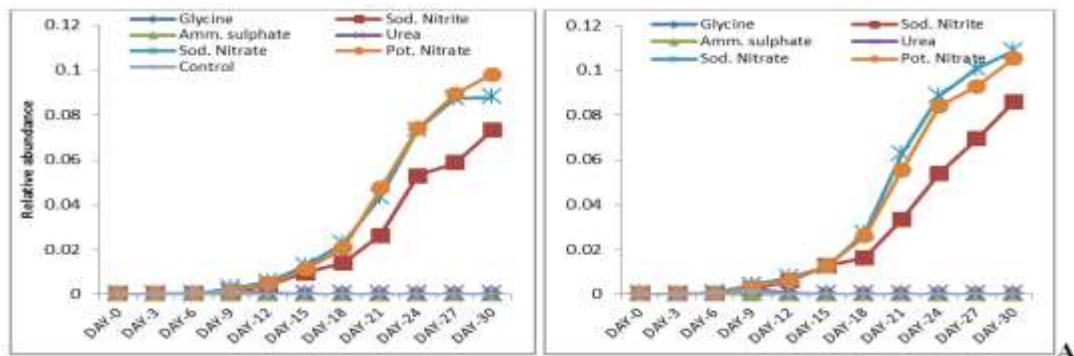


Fig 1: Effect of monochromatic light on relative abundance of A) phycocyanin, B) phycoerythrin and C)



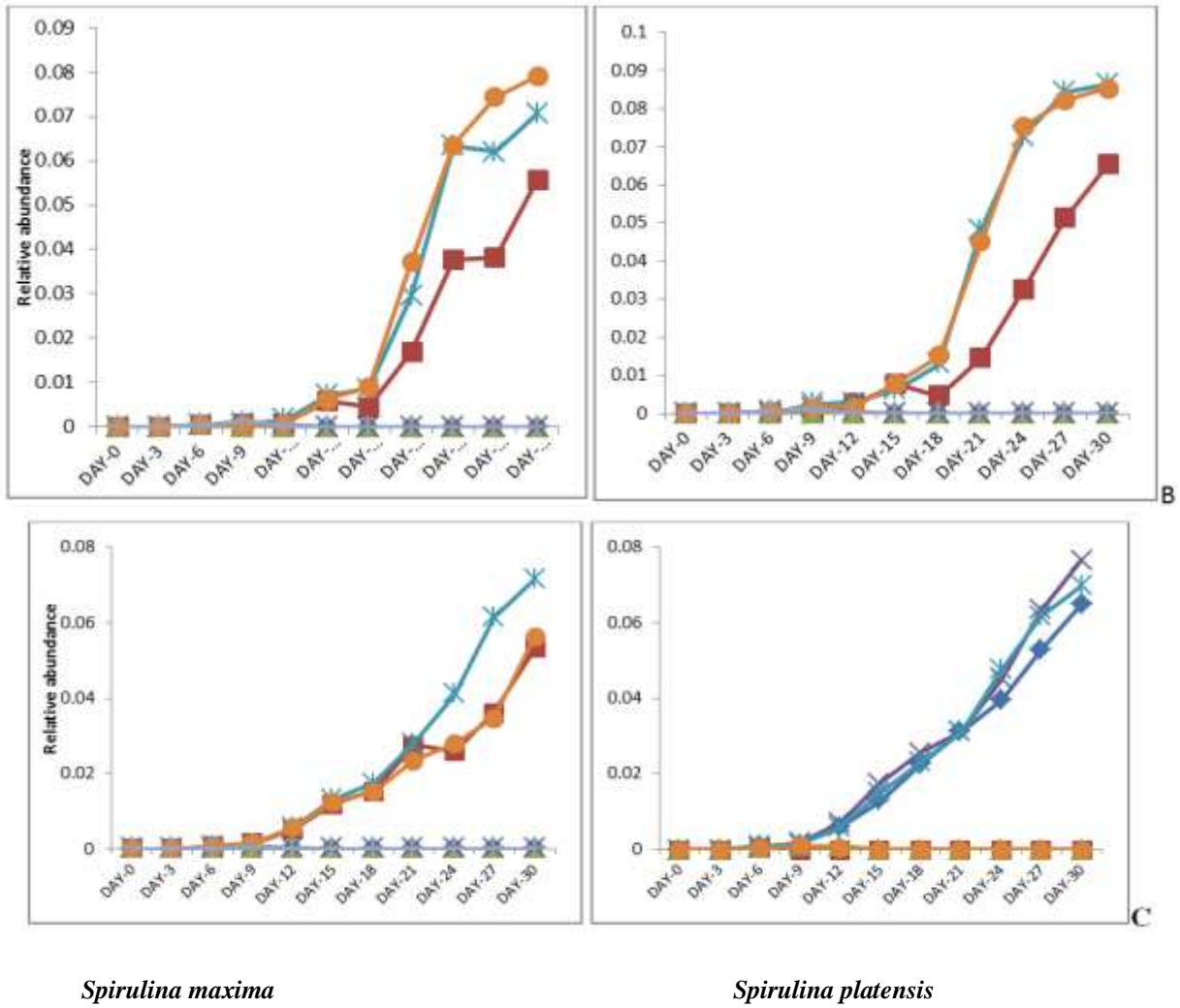
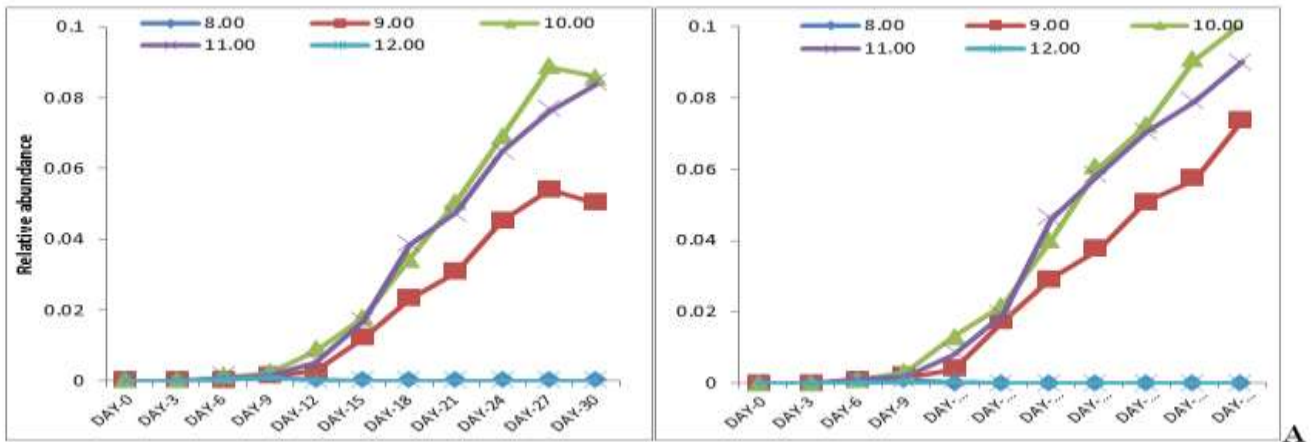


Fig 2: Effect of different nitrogen sources on relative abundance of A) phycocyanin, B) phycoerythrin and C)



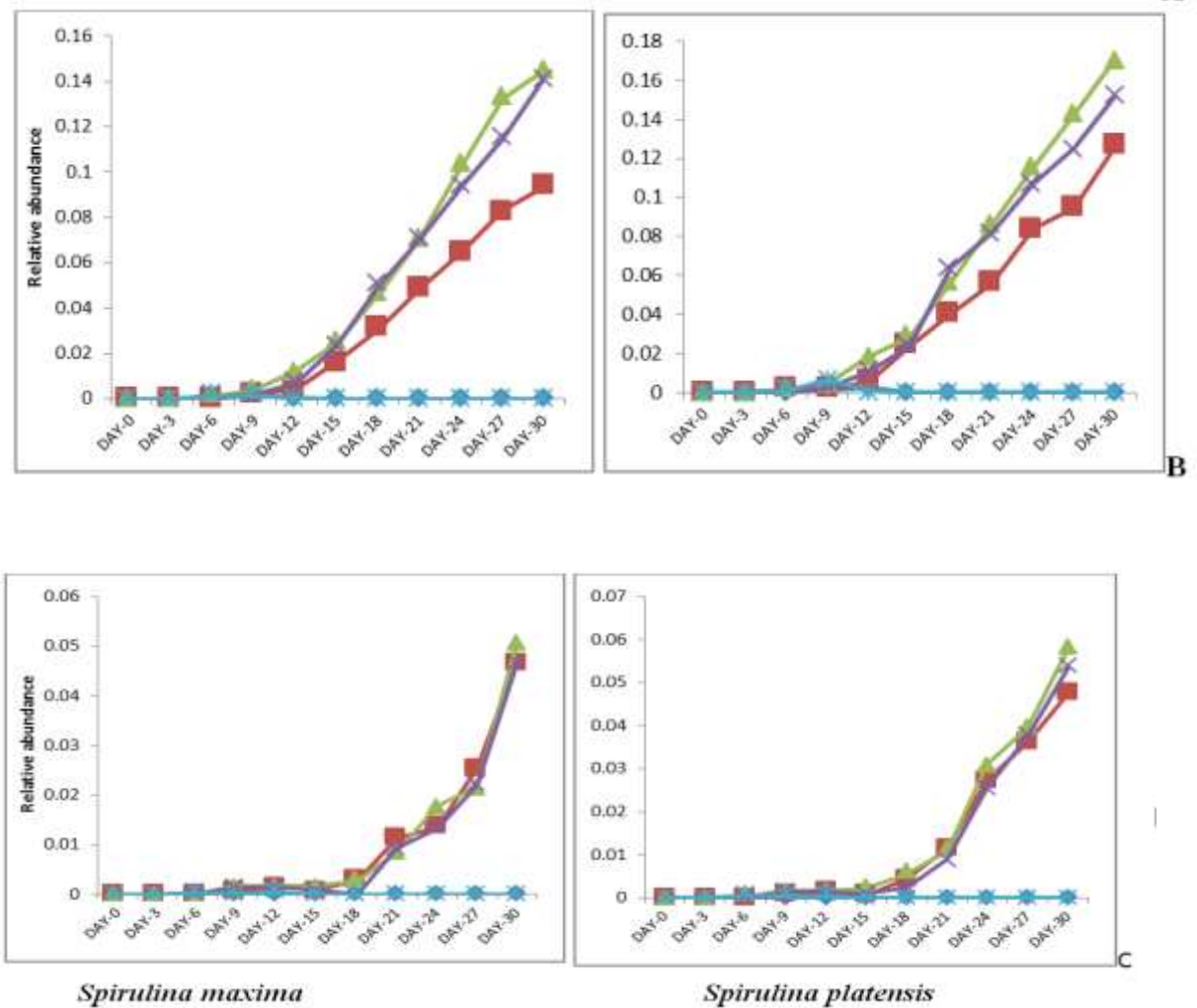


Fig 3: Effect of different pH on relative abundance of A) phycocyanin, B) phycoerythrin, and C) allophycocyanin on *Spirulina maxima* and *S. platensis*.

IV. DISCUSSION

Cyanobacteria are known to be rich source for commercially exploitable value added products these days, and the tropical countries like India, due to its rich biodiversity, can play a pivotal role in this direction. One of such value added products of cyanobacterial origin is phycobiliproteins, components of which are PC, PE and APC, which serve as valuable fluorescent tags with numerous applications, apart from natural pigments (Sekar and Chandramohan, 2008). An added advantage of cyanobacteria cultivation is that all the commercially valuable products can be obtained from single culture and their optimization can be integrated into one single growth cycle (Zeng *et al.*, 2012). Because of these applications, the culture optimization for higher yield has been on the cards for various researchers. *Spirulina* is well studied microorganism at commercial level for phycobiliproteins production. Various researchers have used *S. platensis* (Sala *et al.*, 2018) and *S. maxima* (Castro-García *et al.*, 2018) for the production of phycobiliproteins.

Previously, Fatma (2009) screened 18 cyanobacterial strains including *Spirulina* for potential for phycobiliproteins synthesis. Soundarapandian and Vasanthi (2008) reported highest phycobiliproteins content in *S. platensis* biomass at the end of the cell growth profile (30 days) whereas Kenekar and Deodhar (2014) observed early stationary phase (15 days) as the right time for the harvesting of *Geiterinema sulphureum* biomass for the maximum phycobiliproteins yield.

Various factors such as nutrient availability, salinity, pH, temperature, light irradiance and agitation speed affect the growth and pigments accumulation in cyanobacteria and microalgae (Khazi *et al.*, 2018). Our experiment showed that pink light, followed by red has maximum effect on production of PC, PE and APC in both the cyanobacteria. Similar results were obtained by Kilimtzidi *et al.* (2019), who achieved higher biomass with increased phycocyanin content with red filters. Gupta *et al.* (2018) demonstrated that higher pH and higher nitrate concentration (by sodium nitrate) promotes the concentration of PC in *Spirulina*, which is well supported by our data. The present study is able to demonstrate that production of chlorophyll a, proteins, and phycobiliproteins can be enhanced by using red light filters, higher nitrate concentrations and higher pH of the culture media. Further, *S. platensis* is a better strain as compared to *S. maxima* for production of phycobiliproteins.

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