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Phycobiliprotein production by a novel cold desert cyanobacterium *Nodularia sphaerocarpa* PUPCCC 420.1

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Abstract The present study focuses on finding a good source of phycobiliproteins (PBP). We report a new cyanobacterium *Nodularia sphaerocarpa* PUPCCC 420.1 as a good producer of PBP. The organism produced 445.6 μg PBP mg^{-1} dry biomass. The growth and PBP production of organism were optimized by varying pH of growth medium, nitrogen sources, light quality and sugars. The optimized conditions for PBP production were as follows: pH 8.0, 5 mmol L^{-1} KNO_3, 10 mmol L^{-1} $NaNO_2$, 0.5% sucrose and green light. The PBP production under these conditions ranged from 486 to 676.3 μg mg^{-1} dry biomass. The PBP were more stable when stored in alkaline pH at 4 °C under dark. As per survey of literature, except for *Anabaena fertilissima*, the amount of PBP is significantly higher than the amount of PBP produced by other cyanobacteria. Thus, this organism is a good candidate for the PBP production at commercial level.

Keywords Cyanobacterium · *Nodularia sphaerocarpa* · Optimization · Phycobiliproteins

Introduction

Development of healthy food, free from chemical additives is currently seen as very important. Pigments such as phycobiliproteins, carotenoids and chlorophyll as natural colourants in food are gaining importance over the synthetic ones as they are non-toxic and non-carcinogenic (Chaneva et al. 2007). Production of colourants from cyanobacteria can offer advantages over their extraction from higher plants as they require less space, have a short life cycle and a high rate of biomass production (Hancock 1997).

Phycobiliproteins (PBP) are naturally occurring water-soluble fluorescent pigments produced by cyanobacteria and some eukaryotic algae (Pandey et al. 2013). They serve as accessory or antenna pigments for the photosynthetic light-harvesting complex (Moreno et al. 1995; Sekar and Chandramohan 2008). They may account for up to 60% of cyanobacterial cellular protein and also serve as an additional source of nitrogen reserve in cyanobacteria (Soni et al. 2008). PBP are organized in supramolecular complex called phycobilisomes which are assembled in a regular array on the outer surface of thylakoid membrane and lie adjacent to the photosynthetic reaction centre of PSII in cyanobacteria and red algae (Sidler 1994; MacColl 1998). Based on the spectroscopic properties, these pigments are classified as phycoerythrin (PE, λ_{max} 540–570 nm), phycocyanin (PC, λ_{max} 590–630 nm) and allophycocyanin (APC, λ_{max} 620–655 nm) depending upon their absorption maxima (Ducret et al. 1998; Viskari and Coyler 2002).

PBPs are widely being used as natural colourant in food, nutraceutical and cosmetics industry (Spolaore et al. 2006; Pandey et al. 2013; Sonani et al. 2015). Currently, nutraceutical segment in food industry is booming at 5% per annum and is estimated between 6 billion and 60 billion USD $ in present day global market (Rodríguez-Sánchez et al. 2012). PBP have also been utilized for photodynamic therapy by making use of its function as a photosensitizer (He et al. 1997; Zhang et al. 2000) and may have applications in medicine (Xia et al. 2016). Due to these properties, these pigments are source of attraction for research by scientific communities. Major

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organisms which are exploited for commercial production of phycobiliproteins are limited to cyanobacteria *Arthrospira* (*Spirulina*), *Gleotrichia natans* and the rhodophyte *Porphyridium* (Roman et al. 2002; Spolaore et al. 2006). Since the phycobiliproteins have an array of applications, there is a need to explore more cyanobacteria for large-scale PBP production. Thus, present research was focused on the production and condition optimization of PBP by cyanobacterium *Nodularia sphaerocarpa* PUPCCC 420.1.

Material and methods

The cyanobacterium *Nodularia sphaerocarpa* PUPCCC 420.1 was isolated by our laboratory from cold deserts lake near Koksar village (32°24′30″N; 77°15′5″E) of district Lahaul-Spiti, Himachal Pradesh, India, and raised to axenic cultures through plating technique (Stanier et al. 1971). The cyanobacterium was identified based on morphological characters such as trichome/filament shape, cell dimensions, shape, size and position of heterocyst, presence or absence of sheath using the following monographs (Desikachary 1959; Komárek 2013). The cell size of the cyanobacterium was determined by using an ocular micrometer. The identification was confirmed by 16S rRNA gene sequence (Singh et al. 2014). The pure cultures of the organism were grown in slightly modified Chu-10 medium (Safferman and Morris 1964), where calcium nitrate was replaced with an equimolar amount of calcium chloride. The nutrient medium contained (g L^{-1}) $CaCl_2{\cdot}2H_2O$, 0.232; K_2HPO_4, 0.01; $MgSO_4{\cdot}7H_2O$, 0.025; Na_2CO_3, 0.02; $Na_2SiO_3{\cdot}5H_2O$, 0.044; ferric citrate, 0.0035; citric acid, 0.0035. The cultures were incubated in a culture room at 28 °C ± 2 °C and illuminated for 14 h daily with light intensity of 44.5 μmol photons m^{-2} s^{-1} at the surface of culture vessels.

Condition optimization for phycobiliprotein production

The growth of the organism was monitored at regular interval as an increase in dry weight biomass with time. The experiment was conducted in 250-mL culture flask containing 100-mL culture medium. Exponentially growing cultures, 8 days old, were washed twice with sterilized double distilled water and added in basal medium to give initial absorbance of 0.1 at 720 nm. Ten-microlitre culture was withdrawn, centrifuged at 5000×*g* for 10 min, and the obtained cell pellet washed twice with double distilled water was oven dried at 70 °C for 24 h. The weight of dry biomass was determined. The growth and PBP content were optimized by varying culture conditions such as pH (6.0, 7.0, 8.0 and 9.0) of growth medium, nitrogen sources KNO_3 and $NaNO_2$ (2–15 mmol L^{-1}) and sugars (glucose, fructose, sucrose; 0.5 and 1%). The effect of red light (RL), blue light (BL), green light (GL) and yellow light (YL) on growth and total PBP production was studied by illuminating the culture vessels wrapped with the cellophane papers of respective colours. The irradiance received by the cells inside the flask measured by Digital Luxmeter (Model MS6610) was 14.8, 24.7, 27.1 and 32.1 μmol photons m^{-2} s^{-1} for RL, BL, GL and YL, respectively. The transmission spectra of coloured cellophane papers as measures with a UV-Visible spectrophotometer (Shimadzu, model UV-1280) are given in Fig. 1. At any one time, one parameter was varied keeping others constant.

Extraction and quantification of phycobiliproteins

For extraction of phycobiliproteins, a known volume of homogenous suspension of culture was centrifuged at 5000 rpm for 10 min, and the pellet obtained was resuspended in known volume of water. The contents were then subjected to freeze and thaw cycle till all the pigments were released from the cells. The contents were centrifuged at 5000 x*g* for 10 min, and the absorbance of supernatant was measured at 562, 615 and 652 nm. The total phycobiliprotein (total PBP), phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) were quantified following the equations given by Bennett and Bogorad (1973).

Total protein content was measured according to the method of Lowry et al. (1951). Total carbohydrate was estimated following the methods of Ashwell (1957). To 1 mL of the thick cell suspension of culture, 4 mL of 0.2% anthrone reagent (prepared in concentrated sulphuric acid) was added and thoroughly mixed. The tubes were kept in boiling water bath for 10 min. After this, tubes were allowed to cool at room temperature and absorbance was taken at 540 nm. Standard curve was prepared by using glucose. Total lipids were estimated according to the method of Bligh and Dyer (1959).

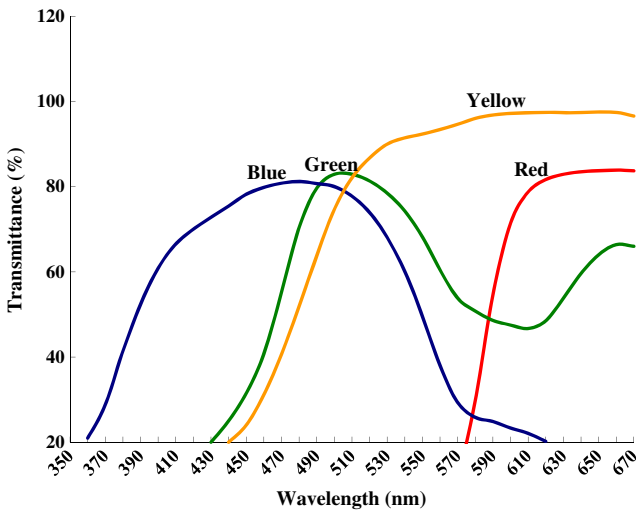

Fig. 1 Transmission spectra of coloured cellophane papers

Stability of phycobiliproteins

Three parameters such as light and dark, temperature (28,18 and 4 °C) and pH (6–9) were chosen to study the stability of crude PBP. A known volume of crude PBP extract was kept under above-mentioned conditions separately keeping one parameter varied and others constant. The absorbance of extract was measured, and time in days for 50% decrease of PBP was determined.

Statistical analysis

All the data are the average of three independent experiments ± standard deviation (SD). Data were statistically analysed by one-way analysis of variance and Tukey's post hoc significance difference test. All statistical analyses were tested against the probability value at 95% confidence level ($p < 0.05$) using GraphPad Prism 6.0 version (www.graphpad.com).

Results

Selection of the test organism

In a preliminary experiment, 20 cyanobacterial species isolated from cold desert area of Lahaul-Spiti, Himachal Pradesh, India, were screened for production of PBP. Among these, the cyanobacterium *Nodularia sphaerocarpa* PUPCCC 420.1 produced maximum amount of PBP equivalent to 44.4% of the dry weight (Table 1). The cyanobacterium is a diazotroph, unbranched and filamentous. The filaments are solitary, straight, bended or spirally coiled with a thick colourless sheath; trichome blue green, cells are short, discoid 4.0 ± 0.8 μm wide and 2.4 ± 0.8 μm long; heterocysts are sub-spherical, 5.0 ± 0.8 μm wide and 2.8 ± 0.4 μm long and are broader than the vegetative cells. The cyanobacterium belongs to order Nostocales of class Cyanophyceae (Fig. 2).

Growth of the microorganism

The pH values of growth medium, nitrogen sources, light quality and sugars were selected to optimize the conditions for growth and phycobiliprotein production. The results revealed that the growth was maximum (160 mg dry biomass L^{-1}) when grown in medium having slightly alkaline pH 8. The supplementation of 5 mmol nitrate L^{-1} (210 mg dry biomass L^{-1}) and 10 mmol nitrite L^{-1} (204 mg dry biomass L^{-1}) in basal medium exhibited maximum growth. Among sugars, 0.5% sucrose in the culture medium supported maximum growth (247 mg dry biomass L^{-1}) of *N. sphaerocarpa*. Among different colours of light, the incubation of the cultures in white light showed more growth, equivalent to 160 mg dry biomass L^{-1} (Fig. 3).

Phycobiliprotein production

The production of PBP under the above-mentioned conditions was also compared. The results revealed that a maximum amount of total PBP (445.6 μg mg^{-1} dry wt. biomass) was produced in the medium with pH 8. *Nodularia sphaerocarpa* produced 486 μg total PBP mg^{-1} dry wt. biomass in the cultures grown under green light. On the other hand, when the cultures were grown in medium with 5 mmol nitrate L^{-1} and 10 mmol nitrite L^{-1}, total PBP production was increased by 47 and 52%, respectively, over the control cultures. The supplementation of 0.5% sucrose in basal medium increased the total PBP production by 42% compared to the control. The increase in total PBP, PC, APC and PE under studied conditions was

Table 1 Comparison of phycobiliprotein content in selected cyanobacteria on day 8

S. No	Organism	Total PBP (% dry wt.)	S. No.	Organism	Total PBP (% dry wt.)
1	*Nodularia sphaerocarpa* PUPCCC 420.1	44.4 ± 2.22	11	*Limnothrix redekei* PUPCCC 116.2	12.7 ± 0.63
2	*Nostoc* sp. PUPCCC 405.2	32.7 ± 1.63	12	*Synechocystis pevalekii* PUPCCC 062.1	11.9 ± 0.59
3	*Nostoc* sp. PUPCCC 405.6	30.6 ± 1.53	13	*Leptolyngbya foveolarum* PUPCCC 112.8	11.2 ± 0.56
4	*Nostoc* sp. PUPCCC 405.8	28.3 ± 1.41	14	*Leptolyngbya lurida* PUPCCC 112.6	10.9 ± 0.54
5	*Pseudanabaena* sp. PUPCCC 106.7	20.2 ± 1.01	15	*Leptolyngbya antarctica* PUPCCC 112.2	10.6 ± 0.53
6	*Phormidium autumnale* PUPCCC 118.4	19.6 ± 0.98	16	*Leptolyngbya benthonica* PUPCCC 112.5	10.1 ± 0.50
7	*Phormidium chalybeum* PUPCCC 118.8	18.3 ± 0.91	17	*Leptolyngbya frigida* PUPCCC 112.1	9.6 ± 0.48
8	*Cyanobium parvum* PUPCCC 007.1	15.2 ± 0.76	18	*Leptolyngbya* sp. PUPCCC 112.7	9.1 ± 0.45
9	*Gloeocapsopsis pleurocapsoides* PUPCCC 008.2	14.6 ± 0.73	19	*Planktothrix clathrata* PUPCCC 108.8	8.8 ± 0.44
10	*Geitlerinema acutissimum* PUPCCC 110.4	13.8 ± 0.69	20	*Planktothrix* sp. PUPCCC 108.6	8.2 ± 0.41

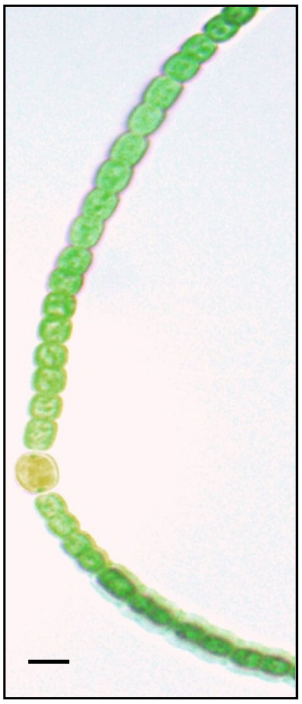

Fig. 2 Photomicrograph of *Nodularia sphaerocarpa* PUPCCC 420.5 (*scale bar* =10 μm)

almost similar, indicating that PC, APC and PE individually contributed to increase in the level of total PBP in this organism (Figs. 4, 5, 6 and 7). The increase in total PBP content of *N. sphaerocarpa* in green light was mainly due to enhancement of PC (17% increase) and APC (12% increase). The supplementation of either 5 mmol nitrate L^{-1}, 10 mmol nitrite L^{-1} or 0.5% sucrose resulted in increase in PC (80–92%) and APC (42–65%) as compared to the control cultures (Figs. 5 and 7). The relative biochemical content of *N. sphaerocarpa* grown under control and optimized condition is presented in

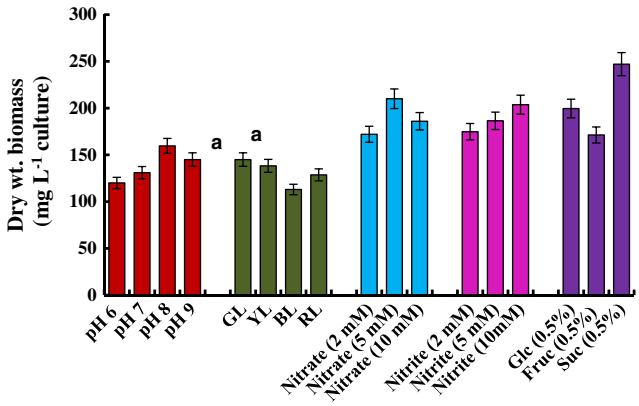

Fig. 3 Growth of *Nodularia sphaerocarpa* on 8 days under varied culture conditions. $n = 9$, *error bar*: SD. Data in *figures with same small letter* are not significantly different from each other at 95% confidence level ($p < 0.05$). Light: green light (GL), yellow light (YL), blue light (BL), red light (RL). Sugars: glucose (Glc), sucrose (Suc), fructose (Fuc)

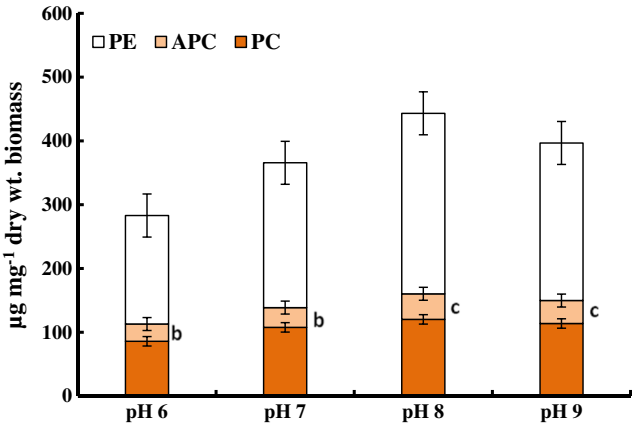

Fig. 4 Comparison of PC, APC> and PE of *Nodularia sphaerocarpa* on 8 days when grown in medium having different pH. $n = 9$, *error bar*: SD. Data in *figures with same small letter* are not significantly different from each other at 95% confidence level ($p < 0.05$)

Fig. 8. The total proteins as well as PBP were enhanced significantly in optimized conditions compared to control conditions.

Stability of phycobiliproteins

Three parameters such as light/dark, temperature and pH were chosen to study the stability of crude PBP extract of *N. sphaerocarpa*. The results revealed that the crude PBP extract was more stable in the dark compared to storage in the light at room temperature. A decrease of 50% in crude PBPs was observed on day 10 in the dark and day 6 in the light (Fig. 9a). The crude PBPs were more stable at 4 °C in dark with a 50% decrease on day 22. There was a 50% decrease in crude PBPs on days 17 and 10 at 18 and 28 °C, respectively (Fig. 9b). PBPs

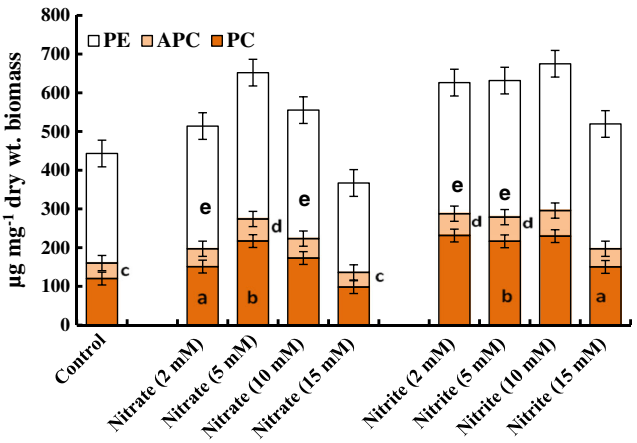

Fig. 5 Comparison of PC, APC and PE of *Nodularia sphaerocarpa* on 8 days when grown in medium having different concentration of potassium nitrate and sodium nitrite. $n = 9$, *error bar*: SD. Data in *figures with same small letter* are not significantly different from each other at 95% confidence level ($p < 0.05$)

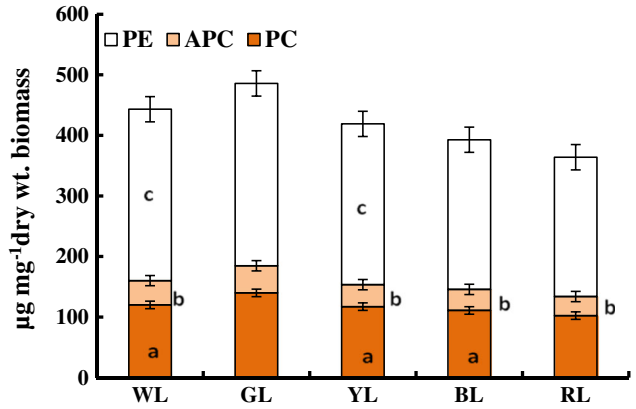

Fig. 6 Comparison of PC, APC and PE of *Nodularia sphaerocarpa* on 8 days when illuminated with different light colours. $n = 9$, *error bar*: SD. Data in *figures with same small letter* are not significantly different from each other at 95% confidence level ($p < 0.05$). Light: white light (WL), green light (GL), yellow light (YL), blue light (BL), red light (RL)

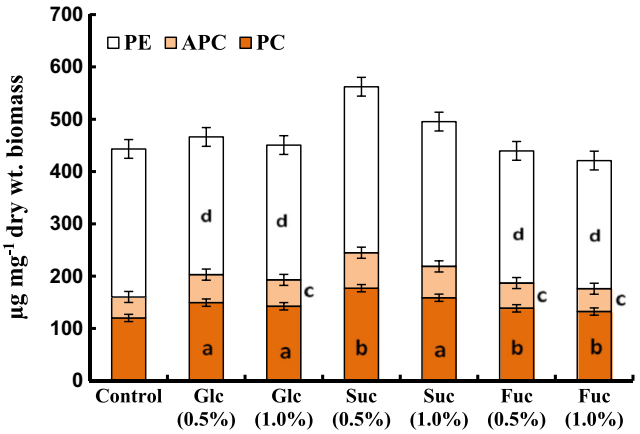

Fig. 7 Comparison of PC, APC and PE of *Nodularia sphaerocarpa* on 8 days when grown in medium having different concentration of sugars. $n = 9$, *error bar*: SD. Data in *figures with same small letter* are not significantly different from each other at 95% confidence level ($p < 0.05$). Sugars: glucose (Glc), sucrose (Suc), fructose (Fuc)

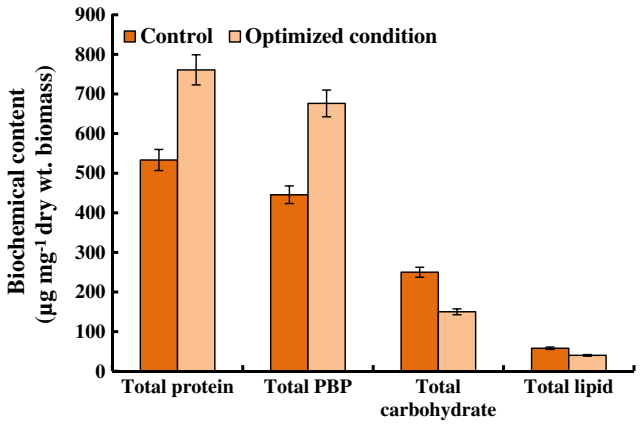

Fig. 8 Comparison of total protein, total PBP, total carbohydrate and total lipids of *Nodularia sphaerocarpa* on 8 days grown in control and optimized conditions. $n = 9$, *error bar*: SD. Data in each parameter of figure at different time interval are significantly different from each other at 95% confidence level ($p < 0.05$)

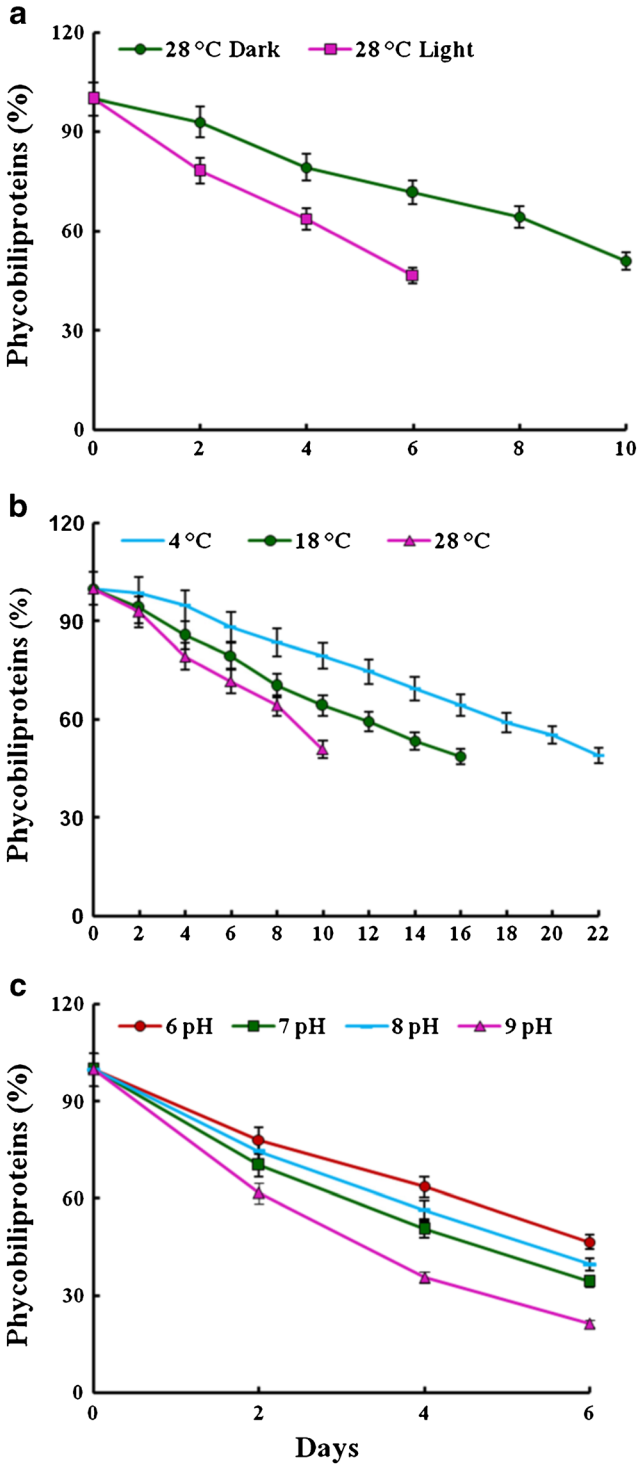

Fig. 9 Effect of light (**a**), temperature (**b**) and pH (**c**) on the stability of phycobiliproteins of *Nodularia sphaerocarpa*. $n = 9$, *error bar*: SD. Data in each line of figure at different time interval are significantly different from each other at 95% confidence level ($p < 0.05$)

were more stable in acidic conditions in the dark with a 53.3% decrease on day 6. In alkaline conditions (pH 9.0), a decrease of 64% in PBP was observed in just 4 days (Fig. 9c).

Discussion

Phycobiliproteins have been reported to have great potential for use in food and pharmaceutical industries (Pandey et al. 2013; Johnson et al. 2014). However, their economically viable production on a commercial scale has been challenging. Inspite of the enormous diversity in algae, only few algal species, *Arthrospira* (*Spirulina*) and *Porphyridium*, are exploited for the production of PBPs (Roman et al. 2002). Thus, there is a need to identify and exploit hyperproducers of PBP. A number of earlier studies were focussed on the PBP production from mesophilic cyanobacteria (Moreno et al. 1995; Hemlata and Fatima 2009; Khattar et al. 2015). During the present study, 20 cyanobacterial species isolated from cold desert of Himachal Pradesh, India, were screened for phycobiliproteins production. Among these, *Nodularia sphaerocarpa* PUPCCC 420.1 produced a high amount of total PBP (44.4% of dry biomass).

It is well known that physical as well as nutrient parameters such as pH of the medium, light quality, nitrogen sources and sugars affected the growth of cyanobacteria (Hemlata and Fatima 2009; Khattar et al. 2015; Tiwari et al. 2015). During the present investigation, these conditions were optimized by taking one parameter at a time. The results revealed that pH of growth medium, supplementation of nitrate and nitrite as nitrogen source or sucrose as sugars significantly affected the growth of *N. sphaerocarpa*. *Nodulatia sphaerocarpa* PUPCCC 420.1 showed maximum growth (159.6 mg dry wt. biomass L^{-1}) in basal medium having pH 8. Similar to our study, the optimum growth of a mesophilic cyanobacterium *Anabaena fertilissima* was reported in alkaline pH 9.5 (Khattar et al. 2015). The growth of *Anabaena* sp. NCCU-9 was maximum in pH range 6–10 (Hemlata and Fatima 2009). Incubation of cultures of *N. sphaerocarpa* PUPCCC 420.1 under different light colours did not support the growth of organism up to the level of growth in control culture under white light (Fig. 3). Our observations are in agreement with Madhyastha and Vatsala (2007) who reported high biomass production in *Spirulina fusiformis* cultures in white light followed by blue and green light. Oberhaus et al. (2007) observed more growth of *Planktothrix agardhii* and *P. rubescens* under white light at 25 °C as compared to other light colours. Blue light enhanced the biomass production in *Pseudanabaena* sp. and *Nanochloris* spp. as compared to white light (Mishra et al. 2012; Vadiveloo et al. 2015, 2016). The high biomass production by *Calothrix elenkinii* was observed in the cultures grown under red light followed by blue and green light (Velu et al. 2015). Better growth of *N. sphaerocarpa* in white light is due to the fact that these received higher irradiances compared to the other colours (Vadiveloo et al. 2015).

The incubation of the cultures in 5 mmol nitrate L^{-1} and 10 mmol nitrite L^{-1} supported maximum growth of *N. sphaerocarpa*. Moore et al. (2002) reported more growth of *Prochlorococcus* and *Synechococcus* sp. in NH_4^+ and urea supplemented medium as compared to NO_3^- supplemented medium. The growth of *Nostoc flagelliforme* was enhanced by 19.8% as compared to the control cultures when grown in medium containing urea as nitrogen source in presence of blue light (Han et al. 2016). Another study found that sodium nitrate as nitrogen source did not support the growth of the cyanobacteria *Nostoc* and *Anabaena* (Simeunovic et al. 2013).

Among various sugars, sucrose (0.5%) proved to be a good source of organic carbon for the growth of *N. sphaerocarpa* compared to fructose and glucose exhibiting 55% more growth in presence of 0.5% sucrose than control cultures. Sugarcane molasses having sucrose has been reported to be the most promising substrate for the production of biomass of *Nostoc* (Borsari et al. 2007). The supplementation of glucose and sucrose in medium stimulated the growth of *Calothrix* and *Anbaena azollae*, respectively (Prasanna et al. 2004, 2006).

Incubation of cultures in green light enhanced the production of total PBP in *N. sphaerocarpa* (Fig. 6). The enhancement of total PBP production in green light is due to increase in PE which may be due to chromatic adaptation (Bezy et al. 2011; Velu et al. 2015). Green light also enhanced the production of PBP in *Fremyella diplosiphon* and *Arthrospira* (*Spirulina*) *platensis* (Oelmuller et al. 1988; Babu et al. 1991). Hemlata and Fatima (2009) observed more PBP in *Anabaena* NCCU-9 under white light. Blue light enhanced the synthesis of phycobiliprotein in *Anabaena ambigua*, *Anabaena fertillissima Westiellopsis iyengarii*, *Spirulina fusiformis* and *Nostoc sphaeroides* (Madhyastha and Vatsala 2007; Vijaya and Anand 2009; Ashok Kumar and Narayanaswamy 2010; Khattar et al. 2015; Ma et al. 2015). These reports suggest that response of cyanobacteria varied with the quality of light. As compared to nitrogen free or NH_4^+ containing medium, the supplementation of 5 mmol nitrate L^{-1} and 10 mmol nitrite L^{-1} resulted in increase of 47–52% in the amount of total PBP of *N. sphaerocarpa*. Our observations are in agreement with Soltani et al. (2007) who have observed high amount of PBP in *Fischerella* grown in nitrate containing medium. Phycobiliproteins serve as nitrogen source, and excess of nitrogen available to cyanobacterial cell may be stored in the form of PBP under nitrogen sufficient conditions. Total PBP in *Nostoc* strain S36 was very high when grown in N_2-free medium, but in *Anabaena* S28, it was high when grown in nitrogen containing medium (Simeunovic et al. 2012).

The supplementation of 0.5% of sucrose increased total PBP by 41% in *N. sphaerocarpa*. Significant increase in total PBP in the sucrose containing cultures of *Anabaena azollae* and *Anabaena fertilissima* has been reported (Prasanna et al. 2006; Khattar et al. 2015). It has been reported that molasses of sugar act as a good substrate for the production of PBP by

Table 2 Comparison of phycobiliprotein production by cyanobacteria under optimized conditions

Organism	Optimized condition	Phycobiliproteins (μg mg^{-1} dry wt. biomass)	Reference
Anabaena fertilissima PUPCCC 410.5	Blue light, sucrose (0.5%)	696.0	Khattar et al. (2015)
Nodularia sphaerocarpa PUPCCC 420.1	Sodium nitrite (10 mM), pH 8, white light	676.0	Present study
Nostoc muscorum	Temperature (30 °C), green light	220.0	Ranjitha and Kaushik (2005)
Anabaena circinalis	White light	202.4	Ojit et al. (2015)
Anabaena NCCC-9	Temperature (30 °C)	127.0	Hemlata and Fatima (2009)
Nostoc sp.	pH 8, Na_2CO_3 (75.48 μM), $NaNO_2$ (17.65 mM), temperature (35 °C), light:dark (16:8 h)	132.0	Johnson et al. (2014)
Arthrospira (Spirulina) plantensis	Urea (2.5 g L^{-1})	199.1	Ajyan et al. (2015)

Nostoc sp. (Borsari et al. 2007). The addition of sucrose in culture medium increased PBP by 30–90% in *A. azollae* (Venugopal et al. 2006). The increase in production of PBS in presence of sucrose may be due to increased energy linked assimilation and ATP production as reported in other cyanobacteria (Prasanna et al. 2004).

Phycocyanin, allophycocyanin and phycoerythrin increased by 17, 12 and 6%, respectively, when cultures of *N. sphaerocarpa* were incubated in green light. The results indicate that cells growing under GL were in a state of high transfer of excitation energy from phycobilisomes/PS-II supercomplex to PS-I. This transfer allowed GL capture by PBS to ultimately drive both PS-I and PS-II photochemistry more efficiently (Campbell 1996; Mishra et al. 2012). The cyanobacterium *Pseudoanabaena* exhibited maximum amount of PE (39 mg L^{-1}) in green light and PC (11 mg L^{-1}) in red light (Mishra et al. 2012). The addition of 5 mmol nitrate L^{-1} or 10 mmol nitrite L^{-1} increased PC, APC and PE content of *Nodularia sphaerocarpa* PUPCCC 420.1 by 34–91%. It has been observed that deficiency of nitrogen resulted in loss of these pigments in cyanobacteria *Oscillatoria splendida* and *Pseudanabaena* sp. (Laura et al. 1987). Addition of 0.5% sucrose in the growth medium resulted in 12–70% increase in PC, APC and PE. Chen and Zhang (1997) observed high production of PC in *Arthrospira plantensis* when it was grown in glucose containing medium. Lebedeva et al. (2005) observed increase in PE and PC content of *Calothrix* sp. with the addition of glucose in growth medium. *N. sphaerocarpa* exhibited maximum production of 120 μg PC mg^{-1} dry biomass, 40 μg APC mg^{-1} dry biomass and 283 μg PE mg^{-1} dry biomass in cultures grown in medium having pH 8. Maurya et al. (2014) observed maximum PBP production by *A. plantensis* at pH 7.0. PBP production was maximum at pH 8 in *Synechocystis* sp., *Gloecapsa* sp., *Anabaena* sp. and *Lyngbya* sp. (Hemlata and Fatima 2009).

Since phycobiliproteins have wide applications, it is important that these proteins remain stable. Thus, stability of crude phycobiliproteins produced by *N. sphaerocarpa* was studied under different condition of light and temperature. The PBP were more stable when incubated in dark at room temperature. The stability of PBP further increased up to 20 days when stored at 4 °C under dark in acidic conditions (pH 6). PBP of *Lyngbya arboricola* were more stable at 4 °C than 25 °C (Tripathi et al. 2007). Antelo et al. (2008) reported that phycocyanin of *A. platensis* was more stable when incubated at temperature 50–55 °C at acidic pH 6. The addition of preservatives may further enhance PBP stability (Kannaujiya and Sinha 2016).

In conclusion, the *N. sphaerocarpa* produced 445.6 mg PBP L^{-1} dry biomass under the control conditions which was enhanced to 653, 676 and 629 mg L^{-1} with the addition of 5 mmol nitrate L^{-1}, 10 mmol nitrite L^{-1} and 0.5% sucrose, respectively. As per available literature, except for *Anabaena fertilissima*, the amount of PBP is significantly higher than the amount of PBP produced by other strains of cyanobacteria (Table 2). Thus, this organism is a good candidate for the phycobiliprotein production at the commercial level. Furthermore, PBP of *N. sphaerocarpa* was more stable when stored in alkaline conditions at 4 °C in the dark.

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