Journal of Applied Phycology (2020) 32:881–888
https://doi.org/10.1007/s10811-019-02012-3

Long-term diazotrophic cultivation of *Trichormus* sp. IMU26: evaluation of physiological changes related to elevated phycobiliprotein content

Mohammed Fadhil Haddad[1,2,3] · Tugba Dayioglu[1,2] · Mustafa Yaman[4] · Barbaros Nalbantoglu[2] · Turgay Cakmak[1]

Received: 27 May 2019 / Revised and accepted: 2 December 2019 / Published online: 2 January 2020

Abstract

A filamentous cyanobacterium, *Trichormus* sp. IMU26, capable of rapid growth and high phycobiliprotein (PBP) production under N_2-fixing conditions was isolated. The strain has been maintained in N-free BG-11 medium for more than 20 months upon isolation. Nitrogen supply resulted in higher soluble protein and saccharide content but a lower growth rate and PBP production in *Trichormus* sp. IMU26. Short-term N-P deprivation induced PBP production with no clear change in growth while growth and PBP content decreased in the longer incubation period. Induction of PBP production in N-P-deprived cells was characterized by FTIR analysis and change in carotenoid-pigment interactions. Rapid induction of zeaxanthin and β-carotene production and slight reduction of echinenone and canthaxanthin levels might be associated with increased PBP levels in short-term N-P deprivation of *Trichormus* sp. IMU26. Overall, long-term diazotrophic growth of N_2-fixing cyanobacteria may increase the PBP yield.

Keywords Nitrogen · Phosphorus · Phycobiliprotein · *Trichormus* sp. · Cyanobacterium

Introduction

Cyanobacteria are an ancient group of Gram-negative, photoautotrophic prokaryotes. They are ubiquitous and abundantly found in all water systems such as freshwater, marine, and thermal springs. They are among the most abundant primary producers on earth and globally responsible for much of the main productivity and nitrogen fixation. (Dvořák et al. 2017). Cyanobacteria produce a wide variety of secondary metabolites, bioactive compounds, and pigments including phycobiliproteins (PBPs) (Li et al. 2019).

Phycobiliproteins are naturally occurring highly fluorescent components of the photosynthetic antenna complexes found in cyanobacteria, red algae and cryptomonads (Li et al. 2019). These water-soluble chromoproteins do not produce visible fluorescence when these proteins are a part of the accessory light-harvesting complex as the excitation energy is transferred to chlorophyll molecules in the photosynthetic membrane. However, once purified, the excitation energy is released as a strong fluorescence (Adir 2005). Therefore, PBPs have been used commercially as fluorescent labels in flow cytometry, fluorescent immunoassays and fluorescence microscopy for diagnostics and biomedical research (Sonani et al. 2016; Zheng et al. 2019). Phycobiliproteins isolated from various cyanobacterial species have been reported to show a variety of pharmacological and health beneficial effects including antioxidant, anticancerous, neuroprotective, anti-inflammatory, hepatoprotective and hypocholesterolemic activities (Fatma 2009). Due to inherent bright color, non-toxic protein nature, easy availability, and potential free radical-scavenging capacity, the PBPs have been employed in food, cosmetics and pharmaceutical industries (Sonani et al. 2016).

The level and composition of PBPs show strain-specific changes in response to environmental fluctuations such as temperature, pH, irradiance, and nutrient availability (Fatma 2009). Among the nutrients, nitrogen and phosphorus are the main elements that limit the development of natural cyanobacteria populations in aquatic ecosystems (Peng et al.

✉ Turgay Cakmak
turgay.cakmak@medeniyet.edu.tr

[1] Department of Molecular Biology and Genetics, Faculty of Science, Istanbul Medeniyet University, 34730 Istanbul, Turkey

[2] Department of Chemistry, Faculty of Arts and Sciences, Yıldız Technical University, 34220 Istanbul, Turkey

[3] Mosul Technical Institute, Northern Technical University, Mosul 41002, Iraq

[4] Department of Nutrition and Dietetics, Istanbul Sebahattin Zaim University, 34700 Istanbul, Turkey

2016). In the current study, four different cyanobacteria isolated from Malıköy-Uyuz thermal spring located in Ankara province in Turkey were evaluated for their PBP production, and some physiological changes in best PBP producing strain was analyzed in relation to nitrogen and phosphorus availability.

Materials and methods

Isolation and identification of the strains

The strains *Trichormus* sp. IMU26, *Anabaenopsis* sp., *Calothrix* sp., and *Nostoc* sp. were isolated from Malıköy-Uyuz thermal spring (40° 34′ 25″ N 32° 39′ 59″ E) located in Ankara province of Turkey. The strains were analyzed for their PBP production capacity and following experiments were conducted on *Trichormus* sp. IMU26 as the best PBP producer. Dynamic parameters such as temperature, salinity, pH, ammonium content, nitrate content, dissolved oxygen, conductivity, and total dissolved particle levels of the water resources were measured by means of a multi-parameter reader (YSI ProPlus, USA) on site when the isolation was accomplished in April 2017.

The strains were identified based on morphological characteristics (Rajaniemi et al. 2005; Waterbury 2006) and genomic information. The strains have been cultured and maintained in Istanbul Medeniyet University Microalgae Culture Collection, Istanbul Medeniyet University, Turkey. In order to obtain genomic information for identification of the strains, the genomic DNA fragment was amplified by PCR, sequenced, and analyzed according to (Lu et al. 1997; Khazi et al. 2018). DNA amplification from genomic DNA containing a partial 16S ribosomal RNA region was performed with PCR by using the following primers: Forward (27F): 5′-AGAGTTTGATCMTGGCTCAG-3′ and Reverse (809R): 5′-GCTTCGGCACGGCTCGGGTCGATA-3′. The same primers were used for Sanger sequencing. Sequence comparison of the 16S rRNA genes was performed using the NCBI databases with BLASTn search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and BioEdit-graphical biological sequence editor v7.0.9.

Preparation of inoculum

The cyanobacteria used in this study are all diazotrophic cyanobacteria showing good growth ability in N-free BG-11 medium (Stanier et al. 1971). After isolation in April 2017, strains have been maintained in N-free BG-11 medium by refreshing them every 15–20 days. Thus, the strains used in this study can be considered acclimated to N deprivation for 20 months until the experimentation. Thereby, cells grown in N-free BG11 medium were used as the experimental reference for PBP production. For experimentation, exponentially growing cells were harvested, washed with distilled water, and used as inoculum (5% v/v). Cyanobacteria were cultivated in 250-mL flasks containing 100 mL of N-free BG-11 medium on a temperature-controlled (27 ± 1 °C) orbital shaker (Sartorius, Certomat BS-T, USA) at 120 rpm under the continuous illumination of 100 μmol photons m^{-2} s^{-1}. For evaluation of P and N availability on PBP production, cultures were either grown in N-replete (refers to complete BG-11 medium, 0.176 mM $NaNO_3$ as N source), N-deficient, or N-P-deficient BG11 growth medium.

Determination of biomass production

The biomass production was determined spectrophotometrically by measuring the chlorophyll-*a* concentration. The concentration of Chl-*a* was calculated according to (Zavřel et al. (2015) with slight modifications. Each sample (2 mL) was centrifuged for 5 min at 10,000× *g* and the pellet was resuspended in 1.5 mL of methanol (100%, precooled to 4 °C), vortexed for 1 min and placed on a rotator for mixing for 20 min under room temperature. Then samples were centrifuged for 5 min at 10,000× *g* and the absorbance of the supernatant was measured at OD_{665} and OD_{720} by using methanol as a blank. The concentration of Chl-*a* was calculated according to the following equation (Ritchie 2006):

$$\text{Chl-}a\ (\text{mg L}^{-1}) = 12.9447\ (OD_{665} - OD_{720}).$$

Quantification of total saccharide and total protein

Each sample (5 mL) was pelleted by centrifugation (2000× *g*, 5 min), washed with sterile water, and centrifuged again (4000× g, 5 min). The pellet was transferred into a 1.5-mL pre-weighed centrifuge tube. After another centrifugation step, the supernatant was completely removed and the remaining cell pellet was first incubated (open cap) in 40 °C in a shaking dry block heater (Thermomixer C, Eppendorf, Germany) for 30 min and weighed for fresh weight determination. Then, the cell pellet was completely dried at 80 °C in a dry block heater overnight. The dry weight of the cells was determined by the weight of the cell tubes subtracted from the dead weight of the tube. Total saccharide and total protein content of the cells were calculated based on the dry weight.

Total saccharide content of the cells were measured by using the modified phenol-sulfuric acid method as described by Zavřel et al. (2018). For protein quantification, lyophilized cell pellets were resuspended in lysis buffer (50 mM Tris–HCl pH 8.0, 2% SDS, 10 mM EDTA, and protease inhibitor mix), subjugated to sonication (3510E-DTH, Branson) for 1 min at 60% power (7 W/pin) and centrifuged at 13,000× *g* at 4 °C. The supernatant was then used for protein determination with bicinchoninic protein assay (He 2011).

Extraction and estimation of phycobiliproteins

Five mL of culture was centrifuged at 4000× *g* for 5 min. The pellet was resuspended in 5 mL sodium phosphate buffer (0.1 M, pH 7.0, containing 1 mM sodium azide) and sonicated (200 W, 30 kHz) for 2 min. After sonication, the samples were kept in the freezer (− 20 °C) for 30 min for freezing and then moved back to room temperature to let samples thaw for another 30 min. The suspension was centrifuged at 4000× *g* for 5 min and the clear supernatant was collected for absorbance measurement. The absorbance of the supernatant was measured at OD_{562}, OD_{620} and OD_{652} for calculation of the phycobiliprotein-PBPs (phycocyanin-PC, allophycocyanin-APC, phycoerythrin-PE) according to the following equations (Bennett and Bogorad 1973):

$$PC\left(mg\ mL^{-1}\right) = \frac{OD_{615} - (0.474 * OD_{650})}{5.34}$$

$$APC\left(mg\ mL^{-1}\right) = \frac{OD_{650} - (0.208 * OD_{615})}{5.09}$$

$$PE\left(mg\ mL^{-1}\right) = \frac{(OD_{562} - (2.41 * PC) - (0.849 * APC))}{9.62}$$

$$\sum PBP = PC + APC + PE$$

Fourier transform infrared spectroscopy

Approximately 50 mg of lyophilized cell biomass was vacuum-dried at 40 °C for 1 h and the dried sample was placed on the sampler module. Infrared spectra were recorded over a wavenumber range of 4000 to 400 cm^{-1} with 128 scans on a Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer-L160000A, USA) equipped with an ATR module. The bands were assigned to specific molecular groups on the basis of biochemical standards and published studies as previously described (Mairet et al. 2011).

HPLC analysis of carotenoids

Quantification of β-carotene, zeaxanthin, echinenone, canthaxanthin, and Chl-*a* in *Trichormus* sp. IMU26 was as described by Chagas et al. (2015) with modifications. The standard calibration curves for β-carotene, lutein, zeaxanthin, canthaxanthin, and Chl-*a* were obtained by using standard stock solutions prepared in methyl tert-butyl ether (MTBE):acetonitrile (50:50, v/v). An Agilent 1200 HPLC-DAD system (Waldbronn, Germany) equipped with a vacuum degasser, autosampler, and a diode-array detector was used to determine the carotenoids in the extracts. The column used was C18 column (Zorbax Eclipse plus) 100 mm × 2.1 mm and 1.8-μm particle size (Agilent Tech, Germany). Before the first measurement, the column was equilibrated for 10 min with 10% eluent A (acetonitrile:water, 90:10 v/v) and 90% eluent B (methanol:water, 85:15 v/v). sThe elution flow rate was set at a constant 1 mL min^{-1} at 25 °C. The mobile phase was acetonitrile/methanol/MTBE, starting with a ratio of 20:75:5 reaching 10:80:10 in 12 min, 0:90:10 in 25 min, 0:75:25 in 40 min, and finally, 0:50:50 in 60 min.

Each experiment was repeated twice with three biological replicates. Thus, the final data in this article are the mean values of at least three separate samples collected at two different times. Means of averages with standard errors are presented throughout the manuscript and data evaluation was done by using *t* tests (two tailed, pair type) with the significance criteria of 0.05 to assess the significance between different groups evaluated for the same time point.

Results

Selection and identification of the best PBP producing strain

The strains *Trichormus* sp. IMU26, *Anabaenopsis* sp., *Calothrix* sp., and *Nostoc* sp. were isolated from Malıköy-Uyuz thermal spring (based on Sanger sequencing information, the strains were registered to NCBI with accession numbers: MK929011 (*Trichormus* sp. IMU26), MK929008 (*Calothrix* sp.), JQ246569 (*Anabaenopsis* sp.,) and MK929009 (*Nostoc* sp.)). Dynamic parameters such as temperature (26.5 °C), salinity (3.25 ppt), pH (6.2), ammonium content (12.1 mg L^{-1}), nitrate content (7.05 mg L^{-1}) and dissolved oxygen (8.7 mg L^{-1}) levels of the water resources were measured by means of a multi-parameter reader (YSI ProPlus, USA) on site when the isolation was accomplished in April 2017.

The strains used in this study have been maintained in N-lacking BG-11 medium for approximately 20 months since they were isolated in April 2017. In order to check their PBP content, the strains were incubated in liquid N-lacking BG-11 growth medium and the PBP contents were measured 6 days after inoculation when they all were in the exponential growth phase. The strain *Trichormus* sp. IMU26 produced the maximum amount of PBP equivalent to 23.2% of dry weight, while it was 4.84%, in *Anabaenopsis* sp., 7.25%, in *Calothrix* sp., and 8.9% in *Nostoc* sp. (Fig.1). The PE content was around 11.8% in *Trichormus* sp. IMU26 while it was not higher than 2.45% in the other strains. The PC content was 6.51% in *Trichormus* sp. IMU26 while it was 2.21% in *Calothrix* sp., 1.92% in *Nostoc* sp., and 1.06% in *Anabaenopsis* sp. Lastly, APC content was 4.87% in *Trichormus* sp. IMU26 while it was 4.53% in *Nostoc* sp., 2.87% in *Calothrix* sp., and 2.48% in *Anabaenopsis* sp. Thus, *Trichormus* sp. IMU26 was selected for further analysis.

The cyanobacterium *Trichormus* sp. IMU26 is a diazotroph, unbranched and filamentous. The filaments are solitary, trichomes are straight, curved, or entangled. Vegetative cells are barrel-shaped 2.5–3.5 μm wide, 3.1–

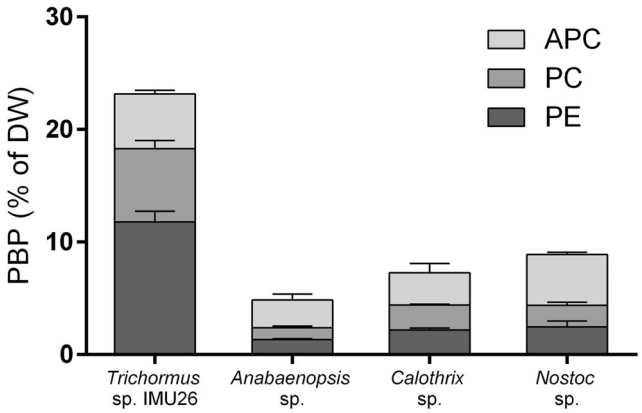

Fig. 1 Phycobiliprotein contents of cyanobacteria maintained in N-free BG-11 medium for 20 months. PBP, phycobiliprotein; PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin. For all data sets, each point represents the mean (± SD) of culture flasks in at least three replicates

4.2 μm long. Heterocytes are solitary, detached or intercalary, rounded or slightly oval, 3.5–4.5 μm wide, 4.5–5.8 μm long. Spores are oval or elliptical, arranged by a few together, without any connection with heterocytes, 2.5–4.5 μm wide, 2.5–5.2 μm long (Fig. 2).

Growth analysis

For experimentation, *Trichormus* sp. IMU26 was grown in N-replete, N-deficient, and N-P-deficient BG11 medium for 16 days of the incubation period. After all treatments, the biomass production was determined spectrophotometrically by measuring the Chl-*a* concentration (Fig. 3). As reflected by changes in Chl-*a* content, N-supply stimulated growth on first days and then suppression of growth was observed after the 6th day of incubation. Approximately 27.6% higher growth was observed on the 4th day of incubation when *Trichormus* sp. IMU26 was grown in N-replete growth medium; however, the growth was suppressed on the following days as characterized with approximately 18.6% decreased growth on day 8 which was followed by a 23.5% decrease

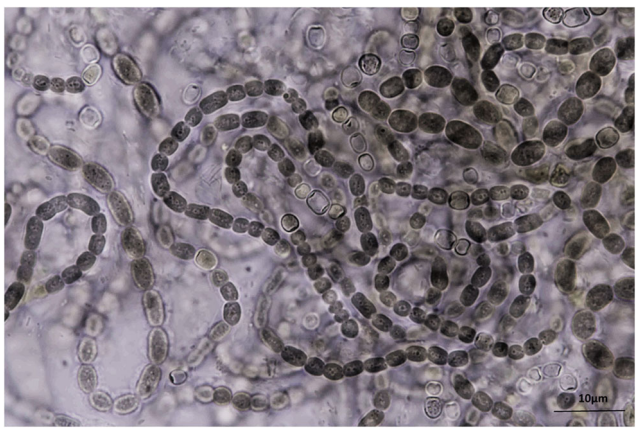

Fig. 2 Microphotograph of *Trichormus* sp. IMU26

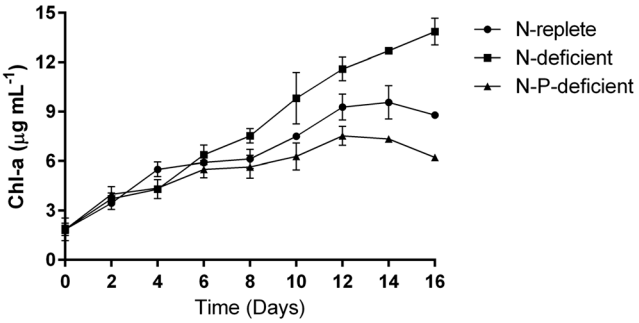

Fig. 3 Growth profile of *Trichormus* sp. IMU26 as reflected by time-dependent changes in Chl-*a* content in 1 mL of cell suspension. Cyanobacteria were incubated in N-replete, N-deficient, and N-P-deficient conditions. Mean (± SD) of culture flasks in at least three replicate

on day 10 and ended up with a 36.5% decreased growth rate at the end of 16 days of incubation when compared with the ones incubated in N-lacking growth medium. On the other hand, N and P deprivation did not cause a significant change in growth on the first days but a remarkable decrease was recorded after the 6th day of growth when compared with the N-deprived ones. A decrease in growth of N- and P-deprived *Trichormus* sp. IMU26 was 24.9% on day 8 and ended up with a 55.2% decreased growth rate. Thus, changes in Chl-*a* content of the respective cultures show that N supply and N-P deprivation caused suppression of growth after the 6th day of incubation and cells entered the stationary phase on the 12th day, while N-deprived cells were exponentially growing with a higher cell density at the end of the 16 days of the incubation period.

Changes in PBP production

Trichormus sp. IMU26 grown in N-replete, N-deficient, and N-P-deficient media were harvested on 6th and 12th days of incubation for PBP analysis (Fig. 4). When compared with N-deprived *Trichormus* sp. IMU26, N supply caused an approximately 26.5% decrease of PBP equivalent production in which 27.9%, 21.9%, and 29.7% decrease in PE, PC, and APC content were observed by the end of 12 days of incubation. On the other hand, N and P deprivation increased overall PBP content up to 18.8% for the first 6 days while a sharp decrease of 49.8% in PBP content was recorded at the end of 12 days of incubation. The increase in PE content was 18.1%, PC content was 21%, and APC content was 17.2% for the first 6 days; however, dramatic decreases in PE, PC, and APC contents were recorded as 48.2%, 47%, and 56.9% respectively by the end of 12 days of incubation.

FTIR analysis

The FTIR bands were assigned to specific molecular groups on the basis of biochemical standards and published studies as previously described (Mairet et al. 2011). The major bands

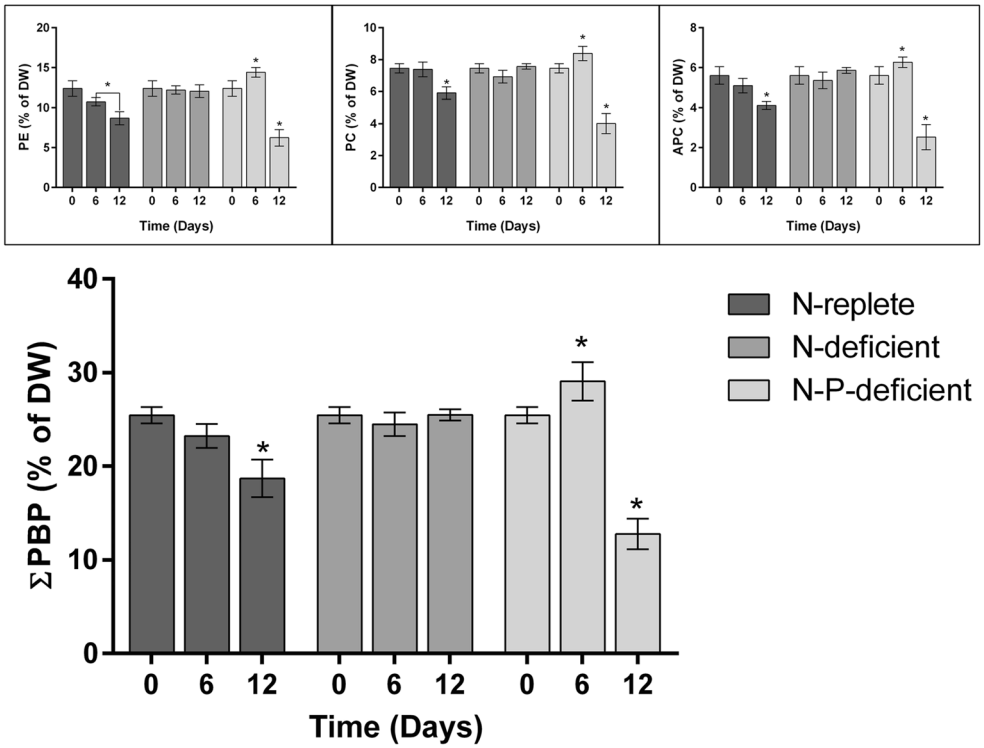

Fig. 4 Phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC), and total phycobiliprotein (PBP) content of *Trichromus* sp. IMU26 in response to N and/or P availability. Mean (± SD) of culture flasks in at least three replicate. Asterisks (*) in the same group indicate a statistically significant difference compared with untreated samples (day 0). *$P < 0.05$

attributed to amides, proteins, lipids, and saccharides are stated in Fig.5. Two major differences were detected in cyanobacteria grown in N-replete and N-P-lacking media. When compared with N-deprived ones, the band 1450 cm^{-1} attributed to C-H stretching of polysaccharides seems to be lost when cyanobacteria were grown in N-replete medium. On the other hand, a new peak 1728 cm^{-1} attributed to C=O stretching of phospholipids emerged when *Trichormus* sp. IMU26 was incubated in N-P-lacking conditions.

Changes in total protein and saccharide content

Total protein and total saccharide content of *Trichormus* sp. IMU26 increased as a response to N supply (Fig. 6a). Approximately 42.4% increased total protein content was recorded at the end of 12 days of incubation while there was 83.1 and 98.5% increase in total saccharide content when N source was supplied to *Trichormus* sp. IMU26. Contrarily, total protein and total saccharide content of

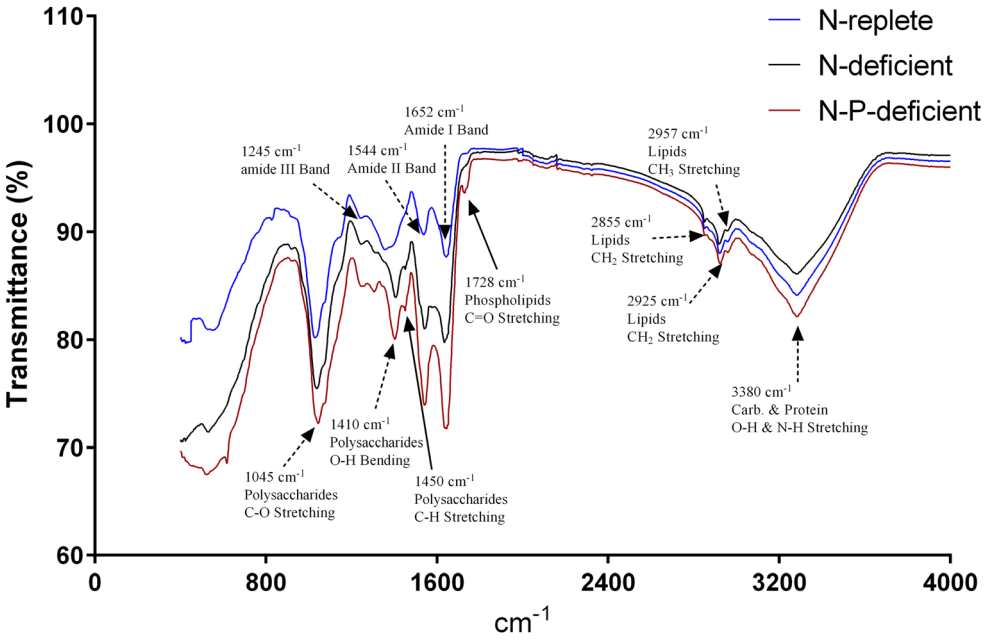

Fig. 5 FTIR spectrum of 6-days old N-replete, N-deficient, and N-P-deficient *Trichormus* sp. IMU26. Dashed arrows show major bands detected in all groups and continuous arrows address specific peaks emerged in N-deprived and N-P-deprived cells

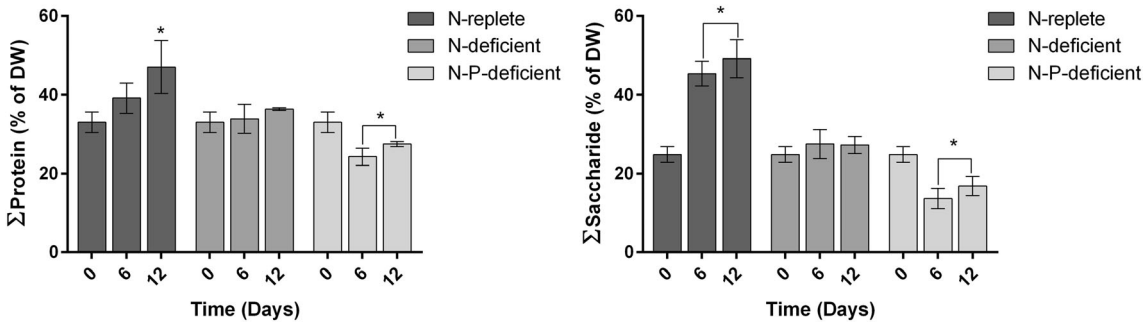

Fig. 6 Change in total soluble protein and saccharide levels in *Trichormus* sp. IMU26. Mean (± SD) of culture flasks in at least three replicate. Asterisks (*) in the same group indicate a statistically significant difference compared with untreated samples (day 0). $*P < 0.05$

Trichormus sp. IMU26 decreased as a response to N-P deprivation (Fig. 6b). There was approximately 26.5 and 16.8% decrease in total protein content while total saccharide content was also recorded as approximately 44.8 and 32% lower than in N-deprived ones as measured on 6th and 12th days of incubation.

Variation in carotenoids production

The β-carotene and zeaxanthin levels increased when the cells were incubated in N-P-deficient medium for 6 days while canthaxanthin and echinenone levels decreased as compared with N-deprived *Trichormus* sp. IMU26 (Fig. 7). Approximately 27% increase in β-carotene/Chl-*a* level was accompanied by a dramatic increase of 209% in zeaxanthin/Chl-*a* production. On the other hand, canthaxanthin/Chl-*a* and echinenone/Chl-*a* levels decreased by 23.5 and 16.2% respectively.

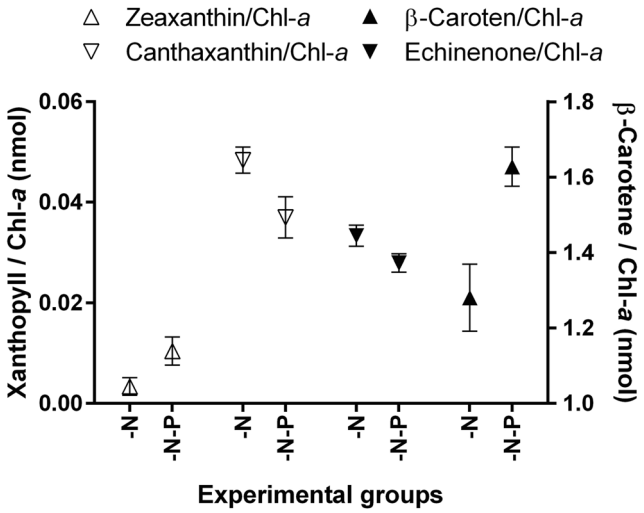

Fig. 7 Change in β-carotene/Chl-*a*, zeaxanthin/Chl-*a*, echinenone/Chl-*a*, and canthaxanthin/Chl-*a* levels in *Trichormus* sp. IMU26 in response to N deprivation, or N-P deprivation. Mean (± SD) of culture flasks in at least three replicates

Discussion

Unlike most other photosynthetic organisms, cyanobacteria are able to photosynthesize efficiently in the low chlorophyll absorption spectrum over the wavelength range 450 to 655 nm by means of PBPs (Glazer and Clark 1986). Phycoerythrin (PE, $\lambda_{Amax} = 540 - 570$ nm; $\lambda_{Fmax} = 575 - 590$ nm), phycocyanin (PC, $\lambda_{Amax} = 610 - 620$ nm; $\lambda_{Fmax} = 645 - 653$ nm), and allophycocyanin (APC, $\lambda_{Amax} = 650 - 655$ nm; $\lambda_{Fmax} = 657 - 660$ nm) are main PBPs in cyanobacteria (Sonani et al. 2016). The overall content and proportions of PBPs differentiate depending on the type of cyanobacterium and fluctuations in the environmental conditions. In this study, *Trichormus* sp. IMU26, *Anabaenopsis* sp., *Calothrix* sp., and *Nostoc* sp. were isolated from the same spring water resource at the same time, and the stock cultures were maintained in N-lacking BG-11 medium for approximately 20 months before the experimentation. Thus, the strains can be considered as N-deficiency acclimated. Analysis of the PBP content of exponentially growing cells showed that *Trichormus* sp. IMU26 produce approximately 4.8-, 3.2-, and 2.6-fold higher PBP equivalent than those in *Anabaenopsis* sp., *Calothrix* sp., and *Nostoc* sp. respectively (Fig.1).

In cyanobacteria, the PBP content and composition is dynamically regulated in response to environmental changes such as light intensity, temperature, pH, and nutrient availability (Fatma 2009). Among the nutrients, N and P are of special importance as mainly N availability affects C and N allocation to PBPs, and P availability affects spore formation in filamentous cyanobacteria (Peng et al. 2016). Phosphorus and N are the main principal nutrients limiting microalgal growth in most water habitats (McCormick et al. 2001). It was reported that the proportion of cyanobacteria increased with distance downstream in a manner related to the dramatic decreases in N and P concentrations that occur in stream water (Mulholland et al. 1995). Thus, in order to evaluate further physiological differences, *Trichormus* sp. IMU26 was incubated in N-replete, N-deficient, and N-P-deficient growth medium. Changes in Chl-*a* content of respective cyanobacteria cultures showed that N supply improved initial growth and N-P

deprivation did not cause a significant change in the first 6 days whereas a substantial decrease of growth in both groups was observed after the 8th day of incubation (Fig.3).

Most recently, Lee et al. (2017) reported that N-free growth of diazotrophic *Nostoc* sp. resulted in higher biomass and PC production. Likewise, Fatma (2009) reported that growing *Anabaena* sp. in N-free medium is superior to nitrate, ammonium, or urea supply in terms of PBP production. Supportively, our results show that 12 days of nitrate supply caused a lower production level of the PBP equivalents when compared with N-deprived *Trichormus* sp. IMU26 (Fig. 4). It seems that long-term diazotrophic growth may increase the PBP yield of N_2-fixing cyanobacteria. Short-term N and P deprivation stimulated higher PBP production but a rapid degradation of PBP equivalents was observed on a longer time period. In fact, the PBPs do not contain phosphorus. The uptake and intracellular accumulation of phosphate are common in filamentous cyanobacteria (Nausch et al. 2009). The phosphate stock is used under P deficiency to support growth and cellular metabolism (Braun et al. 2018). Thus, increased PBP levels in the first 6 days may reflect growth-related photosynthetic adaptation strategies to overcome the absence of P and the diminished PBP levels observed in 12 days of P deprivation might be the consequence of decreased PBP biosynthesis relative to the rate of cell division.

The biomass characteristics in cyanobacteria tend to change as a response to environmental fluctuations. Thus, a potential change in biomass characteristics of *Trichormus* sp. IMU26 grown in N-replete, N-deficient, and N-P-deficient media for 6 days of incubation was evaluated by means of FTIR analysis (Fig.5). The peak at 1450 cm^{-1} attributed to C–H stretching of polysaccharides was visible in N- and N-P-deficient cell biomass; however, it was lost when *Trichormus* sp. IMU26 was grown in N-replete medium. Furthermore, a new peak attributed to C=O stretching of phospholipids (1728 cm^{-1}) emerged when the cyanobacterium was N-P-deprived for 6 days. Phospholipids are the main component of cell membranes, and they maintain a gradient of chemical and electrical processes to ensure cell survival (Singh et al. 2002). Therefore, it seems that there was a reorganization of surface and composition of diazotrophic *Trichormus* sp. IMU26 biomass as a response to N and P deprivation. There was a clear induction of total protein and saccharide production in N-replete *Trichormus* sp. IMU26 while total protein and saccharide levels showed dramatic decreases when the cyanobacterium was grown in N-P-lacking medium (Fig.6). Similarly, Çelekli et al. (2016) reported an extensive change in biomass composition and membrane structure of *Spirulina platensis* as a response to changes in element composition and salinity of the growth medium.

Carotenoids in cyanobacteria serve as light-harvesting pigments in photosynthesis, modulators of membrane microviscosity, and they work against photooxidative damage (Zakar et al. 2016). The strain-specific composition of the carotenoids fluctuates in response to environmental changes such as cell density, light regime, and nutrient composition of the growth medium (Liang et al. 2006). Major carotenoids in cyanobacteria are β-carotene and xanthophylls such as canthaxanthin, echinenone, zeaxanthin, nostoxanthin, and myxoxanthophylls (Takaichi and Mochimaru 2007). Recently, it was reported that carotenoids are required for proper functioning and structural maintenance of PBPs (Tóth et al. 2015). In this study, changes in β-carotene, canthaxanthin, echinenone, and zeaxanthin levels were measured in *Trichormus* sp. IMU26 incubated in N- or N-P-lacking media for 6 days when increased PBP production was observed in N-P-deprived cyanobacteria. There was a remarkable induction of β-carotene and zeaxanthin production accompanied by a decreased level of canthaxanthin and echinenone in N-P-deficient *Trichormus* sp. IMU26 (Fig.7). Zeaxanthin is a hydroxyl derivative of β-carotene while echinenone and canthaxanthin are its keto derivatives. Increased β-carotene and zeaxanthin production in N-P-deprived cells might refer to the protection of pigment systems from photoinhibition and oxidative stress. Recently, Kusama et al. (2015) showed that zeaxanthin and echinenone protect the repair part of the PSII recovery cycle from photoinhibition via reactive oxygen-scavenging activities. Our results showed that there was approximately a 209% increase in zeaxanthin production while only a 16.2% decrease in echinenone production in N-P-deprived cells (Fig. 7). On the other hand, Gruszecki and Strzałka (2005) reported that zeaxanthin, a polar carotenoid, induces membrane rigidity while echinenone may have a neutral or fluidizing effect. Therefore, the new peak attributed to C=O stretching of phospholipids (1728 cm^{-1}) observed in FTIR analysis of N-P-deprived *Trichormus* sp. IMU26 (Fig.5) may refer to the membrane modulation activity of carotenoids in filamentous cyanobacteria. Lastly, blue-green light-absorbing orange carotenoid protein (OCP) in cyanobacteria is known to act as a stress sensor and energy quencher which is able to bind phycobilisomes when it is activated (Punginelli et al. 2009). Activation or deactivation of OCP necessitates a dynamic adjustment in carotenoid-chlorophyll and carotenoid-bilin interactions (Thurotte et al. 2015). Thus, elevated PBP levels in response to 6-day N-P deprivation of *Trichormus* sp. IMU26 may be due to induced β-carotene and zeaxanthin production accompanied by a slight decrease in echinenone and canthaxanthin levels.

As a conclusion, this study introduces a filamentous diazotrophic cyanobacterium, *Trichormus* sp. IMU26, capable of rapid growth and increased PBP production when deprived of N. Moreover, short-term N-P deprivation induces the PBP production while biomass production is not affected. Lastly, rapid induction of zeaxanthin and β-carotene production might be associated with increased PBP production in cyanobacterium *Trichormus* sp. IMU26 cultivated in N-P-free growth medium.

Author contributions M.H. and T.D performed the research, M.Y contributed to HPLC analysis, B.N supervised, and T.C designed the research and wrote the paper.

Funding information This study was supported by a grant from the Turkish Ministry of Agriculture and Forestry General Directorate of Agricultural Research (TAGEM-12/AR-GE/13).

References

Adir N (2005) Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant. J Photosynth Res 85:15–32

Bennett A, Bogorad L (1973) Complementary chromatic adaptation in a filamentous blue-green alga. J Cell Biol 58:419–435

Braun PD, Schulz-Vogt HN, Vogts A, Nausch M (2018) Differences in the accumulation of phosphorus between vegetative cells and heterocysts in the cyanobacterium *Nodularia spumigena*. Sci Rep 8: 5651

Çelekli A, Topyürek A, Markou G, Bozkurt H (2016) A multivariate approach to evaluate biomass production, biochemical composition and stress compounds of *Spirulina platensis* cultivated in wastewater. J Appl Biochem 180:728–739

Chagas A, Rios A, Jarenkow A, Marcilio N, Ayub M, Rech R (2015) Production of carotenoids and lipids by *Dunaliella tertiolecta* using CO_2 from beer fermentation. Process Biochem 50:981–988

Dvořák P, Casamatta DA, Hašler P, Jahodářová E, Norwich AR, Poulíčková A (2017) Diversity of the cyanobacteria. In: Hallenbeck P (ed) Modern topics in the phototrophic prokaryotes. Springer, Cham, pp 3–46

Fatma T (2009) Screening of cyanobacteria for phycobiliproteins and effect of different environmental stress on its yield. Bull Environ Contam Toxicol 83:509–515

Glazer A, Clark J (1986) Phycobilisomes: macromolecular structure and energy flow dynamics. Biophys J 49:115–116

Gruszecki WI, Strzałka K (2005) Carotenoids as modulators of lipid membrane physical properties. Biochim Biophys Acta -Molec Basis Disease 1740:108–115

He F (2011) BCA (bicinchoninic acid) protein assay. Bio-protocol 1:1–2

Khazi MI, Demirel Z, Dalay MC (2018) Evaluation of growth and phycobiliprotein composition of cyanobacteria isolates cultivated in different nitrogen sources. J Appl Phycol 30:1513–1523

Kusama Y, Inoue S, Jimbo H, Takaichi S, Sonoike K, Hihara Y, Nishiyama Y (2015) Zeaxanthin and echinenone protect the repair of photosystem II from inhibition by singlet oxygen in *Synechocystis* sp. PCC 6803. Plant Cell Physiol 56:906–916

Lee NK, Oh H-M, Kim H-S, Ahn C-Y (2017) Higher production of C-phycocyanin by nitrogen-free (diazotrophic) cultivation of *Nostoc* sp. NK and simplified extraction by dark-cold shock. Bioresour Tecnol 227:164–170

Li W, Su H-N, Pu Y, Chen J, Liu L-N, Liu Q, Qin S (2019) Phycobiliproteins: molecular structure, production, applications, and prospects. Biotechnol Adv 37:340–353

Liang C, Zhao F, Wei W, Wen Z, Qin S (2006) Carotenoid biosynthesis in cyanobacteria: structural and evolutionary scenarios based on comparative genomics. Int J Biol Sci 2:197–207

Lu W, Evans EH, McColl SM, Saunders VA (1997) Identification of cyanobacteria by polymorphisms of PCR-amplified ribosomal DNA spacer region. FEMS Micrbiol Lett 153:141–149

Mairet F, Bernard O, Masci P, Lacour T, Sciandra A (2011) Modelling neutral lipid production by the microalga *Isochrysis galbana* under nitrogen limitation. Bioresour Technol 102:142–149

McCormick PV, O'Dell MB, Shuford RB III, Backus JG, Kennedy WC (2001) Periphyton responses to experimental phosphorus enrichment in a subtropical wetland. Aquat Bot 71:119–139

Mulholland PJ, Marzolf ER, Hendricks SP, Wilkerson RV, Baybayan AK (1995) Longitudinal patterns of nutrient cycling and periphyton characteristics in streams: a test of upstream-downstream linkage. J North Am Benthol Soc 14:357–370

Nausch M, Nausch G, Lass HU, Mohrholz V, Nagel K, Siegel H, Wasmund N (2009) Phosphorus input by upwelling in the eastern Gotland Basin (Baltic Sea) in summer and its effects on filamentous cyanobacteria. Estuar Coast Shelf Sci 83:434–442

Peng G, Fan Z, Wang X, Chen C (2016) Photosynthetic response to nitrogen source and different ratios of nitrogen and phosphorus in toxic cyanobacteria, *Microcystis aeruginosa* FACHB-905. J Limnol 75:560–570

Punginelli C, Wilson A, Routaboul J-M, Kirilovsky D (2009) Influence of zeaxanthin and echinenone binding on the activity of the orange carotenoid protein. Biochim Biophys Acta Bioenerg 1787:280–288

Rajaniemi P, Hrouzek P, Kaštovska K, Willame R, Rantala A, Hoffmann L, Komárek J, Sivonen K (2005) Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, cyanobacteria). Int J Systemat Evol Microbiol 55:11–26

Ritchie RJ (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynth Res 89:27–41

Singh SC, Sinha RP, Häder D-P (2002) Role of lipids and fatty acids in stress tolerance in cyanobacteria. Acta Protozool 41:297–308

Sonani RR, Rastogi RP, Patel R, Madamwar D (2016) Recent advances in production, purification and applications of phycobiliproteins. World J Biol Chem 7:100–109

Stanier RY, Kunisawa R, Mandel M Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order Chlorococcales). Bact Rev 35:171–201

Takaichi S, Mochimaru MJC (2007) Carotenoids and carotenogenesis in cyanobacteria: unique ketocarotenoids and carotenoid glycosides. Cell Mol Life Sci 64:2607–2619

Thurotte A, Lopez-Igual R, Wilson A, Comolet L, de Carbon CB, Xiao F, Kirilovsky D (2015) Regulation of orange carotenoid protein activity in cyanobacterial photoprotection. J Plant Physiol 169:737–747

Tóth TN, Chukhutsina V, Domonkos I, Knoppová J, Komenda J, Kis M, Lénárt Z, Garab G, Kovács L, Gombos Z (2015) Carotenoids are essential for the assembly of cyanobacterial photosynthetic complexes. Biochim Biophys Acta Bioenerg 1847:1153–1165

Waterbury JB (2006) The cyanobacteria—isolation, purification and identification. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 1053–1073

Zakar T, Laczko-Dobos H, Toth TN, Gombos Z (2016) Carotenoids assist in cyanobacterial photosystem II assembly and function. Front Plant Sci 7:295

Zavřel T, Sinetova MA, Červený J (2015) Measurement of chlorophyll a and carotenoids concentration in cyanobacteria. Bio-protocol 5: 1467

Zavřel T, Očenášová P, Sinetova MA, Červený J (2018) Determination of storage (starch/glycogen) and total saccharides content in algae and cyanobacteria by a phenol-sulfuric acid method. Bio-protocol 8: e2966

Zheng Y, Mo L, Zhang W, Duan Y, Huang J, Chen C, Gao Y, Shi X, Li F, Yang J, Guo Y (2019) Phycocyanin fluorescent probe from *Arthrospira platensis*: preparation and application in LED-CCD fluorescence density strip qualitative detection system. J Appl Phycol 31:1107–1115