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MICROBIOLOGY

Effects of light quality and intensity on phycobiliprotein productivity in two *Leptolyngbya* strains isolated from southern Bahia's Atlantic Forest

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Abstract: Cyanobacterial phycocyanin and phycoerythrin are gaining commercial interest due to their nutrition and healthcare values. This research analyzed the biomass accumulation and pigment production of two strains of *Leptolyngbya* under different combinations of light colors and intensities. The results showed that while *Leptolyngbya* sp.4 B1 (B1) produced all phycobiliproteins, *Leptolyngbya* sp.5 F2 (F2) only had phycocyanin and allophycocyanin. Both the color of the light and its light intensity affect the biomass accumulation and phycoerythrin concentration in strain B1. Although white light at medium intensity (50 µmol m^{-2} s^{-1}) causes greater biomass accumulation (1.66 ± 0.13 g_{DW} L^{-1}), low-intensity (25 µmol m^{-2} s^{-1}) green light induces lower biomass accumulation with twice the pigment content (87.70 ± 2.46 mg g_{DW}^{-1}), culminating in 71% greater productivity. In contrast, for the F2 strain, light intensity positively influenced biomass and pigment accumulation, being observed 2.25 ± 0.10 g_{DW} L^{-1} under white light at 100 µmol m^{-2} s^{-1} and higher phycocyanin concentration (138.38 ± 3.46 mg g_{DW}^{-1}) under red light at 100 µmol m^{-2} s^{-1}. These findings provide insights into optimizing the growth conditions by altering the intensity and wavelength of light for future production of phycocyanin and phycoerythrin from local cyanobacteria.

Key words: Biodiversity, light effects, phycocyanin, phycoerythrin.

INTRODUCTION

Phycocyanin (PC) and phycoerythrin (PE) are phycobiliproteins founded in cyanobacteria and some macro and microalgae, and typically characterized by intense blue and red or purple color, respectively (Limrujiwat et al. 2022). These water-soluble fluorescent chromoproteins are responsible for absorbing sunlight at wavelengths that are inefficiently absorbed by chlorophylls (Pagels et al. 2019). Phycocyanin and phycoerythrin have been described as having anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, and anti-hyperglycemic properties, and are used as an adjuvant in radiotherapy, biochemical markers, food colorings, and pigments for lipsticks and eyeshadows (Kefayat et al. 2019, Hsieh et al. 2021, Wu et al. 2016, Galetovic et al. 2020, Moraes & Kalil 2018, Hamed 2016). Additionally, phycoerythrin has properties that can help reduce the effects of aging and oxidative stress on the skin (Patel et al. 2018, Pagels et al. 2019). According to Verified Market Research, the global phycocyanin market already accounts for around US$ 173.29 million, with prospects for growth at an annual rate of 9.7%, reaching US$ 364.16 million in 2030. Around 60% of this volume corresponds to the beverage and food sectors. On the other hand, according to Research Report World, phycoerythrin has a discrete market of US$ 14.79 million in 2022 and prospects of

reaching US$ 20.67 million in 2028. The main use of phycoerythrin is in research and development as an immunofluorescence, flow cytometry, or cell staining marker, and it can cost more than US$ 1500 per pure milligram (Zittelli et al. 2022).

Despite belonging to the same class of pigments, phycocyanin and phycoerythrin from different species may vary in color, physicochemical properties, and biological activity. This is why several studies have sought new phycobiliproteins and optimized their production (Sun et al. 2009, Kannaujiya et al. 2017). Recently, our group isolated two strains of *Leptolyngbya* from the Atlantic Forest in the microregion of Porto Seguro, in the south of Bahia (Brazil), with high contents of phycoerythrin and phycocyanin (E. S. Gallina et al., unpublished data, Vaz et al. 2015). This was the first reference for freshwater cyanobacteria from that region and one of their biotechnological potentials. Although they presented increased pigment contents (between 5 and 7% of dry weight; 5.69 mg L^{-1} d^{-1} and 6.53 mg L^{-1} d^{-1} for phycoerythrin and phycocyanin, respectively) when compared to many strains, the productivity was still lower than that obtained in commercial strains such as *Arthrospira platensis* (100 mg L^{-1} d^{-1} of phycocyanin) or *Porphyridium purpureum* (around 10 mg L^{-1} d^{-1} of phycoerythrin), requiring the optimization of cultivation conditions to be competitive (Li et al. 2022, Chaiklahan et al. 2022). Considering that sustainable use of the local biodiversity is an important asset for the consolidation of the bioindustry and the establishment of a sustainable development model in the country, the production of high-added-value products in a CO_2-mitigating cultivation system is an alternative to linking economic development to climate action (Gallina et al. 2017, Griggs 2013).

The growth of cyanobacteria, as well as their biomass chemical composition and pigmentation, can be impacted by process parameters such as light color and intensity, temperature, nutrient availability, and photobioreactor characteristics (Hsieh-Lo et al. 2019). The color of light has potential to modulate the composition of the light-harvesting system of cyanobacteria, while the proper adjustment of the light intensity can affect the productivity of different phycobiliprotein (Khazi et al. 2021, Klepacz-Smółka et al. 2020). However the effects is distinct of light color and intensity are different according to the strain that is used. Results reported by Xie et al. (2015) show that *Arthrospira platensis* WH879 can range maximum phycocyanin productivity of 94.8 mg L^{-1} d^{-1} at continuous 300 µmol photons m^{-2} s^{-1} light intensity, with a total dry biomass accumulation of up to 8 g_{DW} L^{-1} at the end of 13 days of cultivation and 16.1% of phycocyanin content in relation to dry biomass. On the other hand, Chaiklahan et al. (2022) reported maximum phycocyanin productivity (123 mg L^{-1} d^{-1}) at 2300 µmol photons m^{-2} s^{-1} for *Arthrospira platensis* BP, after 14 days of cultivation, with a total dry biomass accumulation around 8,6 g_{DW} L^{-1}, and a phycocyanin content of almost 20%. The same occurs with different light colors, while Ho et al. (2018) reported maximum phycocyanin productivity (101.1 mg L^{-1} d^{-1}, total biomass of 8,2 g_{DW} L^{-1} after 12 days of cultivation, and 14.9% of phycocyanin) at white light for *Arthrospira platensis*. Lee et al. (2017) reported 57,4 mg L^{-1} d^{-1} at red light for *Nostoc* sp. NK (total biomass around 3,2 g_{DW} L^{-1} after 10 days of cultivation, with 18% (w/w) of phycocyanin content). Prates et al. (2018) reported almost 19 mg L^{-1} d^{-1} of phycocyanin at green light for *Spirulina platensis* LEB18, compared with almost 10 mg L^{-1} d^{-1} at red light. For phycoerythrin production, green and white colors at low intensities results in higher pigment content (Ojit et al. 2015, Mishra et al. 2012)

Numerous studies have explored the effects of light color and intensity on phycobiliprotein production (Pagels et al. 2020, Lin et al. 2022, Khan et al. 2019), typically in combination with other factors such as photoperiod and medium composition. However, few studies have examined the

combined effects of those parameters. Since efficient production of photosynthetic pigments, such as phycobiliproteins (PE and PC), is closely associated with chromatic acclimatization, optimizing light color is of paramount importance. Likewise, light color and intensity can affect biomass yields and pigment accumulation. The present study aims to investigate the effects of different combinations of light colors (white, green, red, and blue) and light intensities (25, 50, and 100 µmol photons m^{-2} s^{-1}) on biomass accumulation, phycobiliprotein content, and volumetric productivities in two strains of *Leptolyngbya sensu lato*.

MATERIALS AND METHODS

Cyanobacterial strains

Cyanobacteria strains were isolated from two freshwater reservoirs inserted in preserved areas of Atlantic Forest, Santa Cruz Cabrália, Bahia, Brazil (Reservoir 1: 16° 22' 40,5" S × 39° 11' 22,0" W; Reservoir 2: 16° 21' 23,1" S × 39° 11' 19,9" W). AD406cc biodiversity access register, according to the Brazilian Biodiversity Law (Brazil 2015). *Leptolyngbya* strains were isolated and confirmed as unialgal cultures using a combination of micropipette picking and spread plate techniques. The liquid and solid (1% w/v) forms of BG-11 medium, supplemented with 50 µg L^{-1} of cycloheximide (Sigma Aldrich), were used for the isolation and storage of the strains (Rippka et al. 1979). The strains were maintained at 25 °C in 12-hour light-dark cycles with a light intensity of 20 µmol photons m^{-2} s^{-1}. These two strains have been characterized and identified as *Leptolyngbya* sp.5 F2 (F2) (Reservoir 1) and *Leptolyngbya* sp.4 B1 (B1) (Reservoir 2), using a morphological and phylogenetic approach (E.S. Gallina et al., unpublished data). Despite presenting morphological characteristics close to those considered diagnostic for a complex of genera, such as *Monilinema* (Malone et al. 2021), *Pantanalinema*, *Alkalinema* (Vaz et al. 2015) the strains are phylogenetically distinct from each other and not related to the type species of these genera. In addition, the isolated strains differ in ecological aspects, since all these genera were described for alkaline environments. Considering their potential to represent new species or genera, these strains are identified as *Leptolyngbya sensu lato*.

Experimental setup

To study phycobiliprotein responses to different light quality (color) and intensity and for simultaneous execution of the conditions tested for each strain, the structure schematized in Figure 1 was built. While in the cultivation of *Leptolyngbya* sp.5 F2, white, blue, and red lights were used (Figure 1a), for *Leptolyngbya* sp.4 B1, white, green, and red lights were used (Figure 1b). This difference is because the F2 strain produces more phycocyanin and the B1 strain produces more phycoerythrin (E.S. Gallina et al., unpublished data). The luminous intensities employed were 25, 50, and 100 µmol photons m^{-2} s^{-1} (approx.) for all light colors. The colorful lamps used were from AAA-Top (tubular LED T8, 30 cm, collor, 5 W), while the white lamps were from Granfei (Linear 30cm, 4.5 W, and 640 lumens). The use of different lamps was necessary for the light intensity to be the same in all cases. The experiment was executed in 500 mL Erlenmeyer flasks, filled with 270 mL of BG-11 medium, and inoculated with 30 mL containing 0.50 g_{DW} L^{-1} (approx.) of biomass from individual cyanobacterial strains – providing an initial biomass concentration of 0.05 g_{DW} L^{-1} (approx.). The agitation was due to the injection of filtered atmospheric air, and the photoperiod was of 16:08 h (light:dark). The cultivation was maintained for

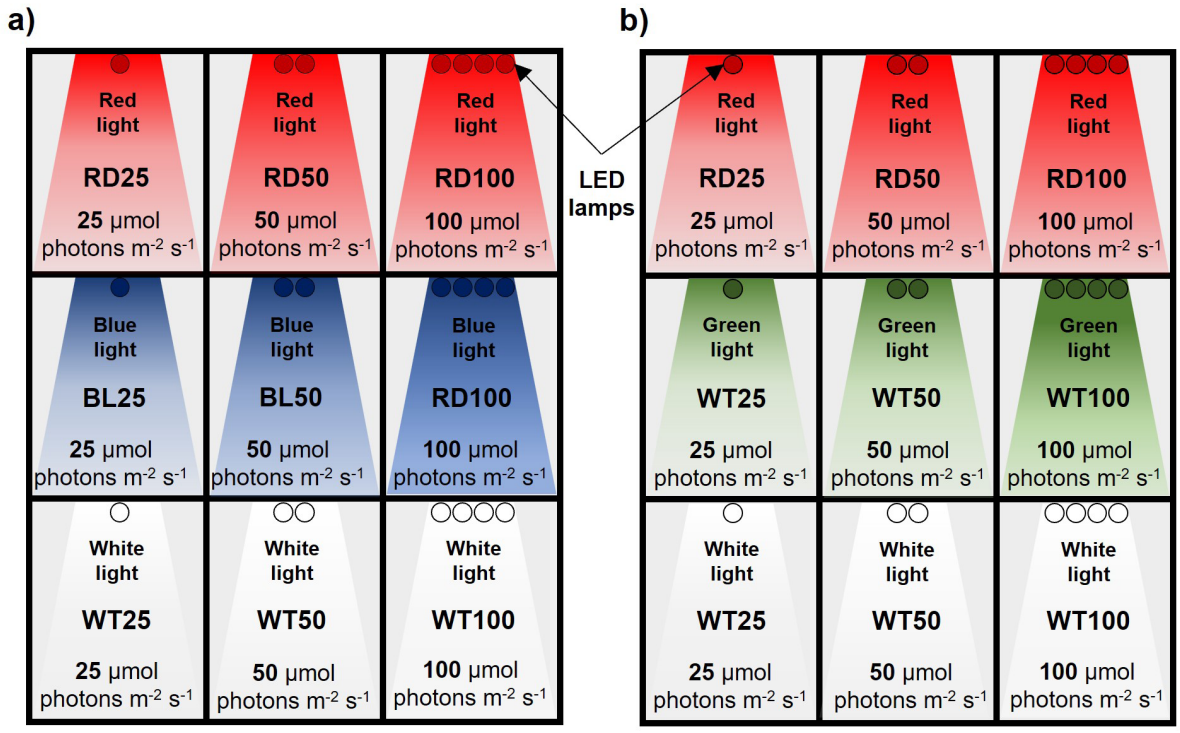

Figure 1. Scheme of the structure and experiment to evaluate the influence of light quality and light intensity on the production of pigments and biomass of *Leptolyngbya* sp.5 F2 (a) and *Leptolyngbya* sp.4 B1 (b). The treatments are represented by the abbreviation of the color (WT: White; BL: Blue; GR: Green; RD: Red) followed by the light intensity (25, 50 or 100 µmol photons m^{-2} s^{-1}).

14 days at a temperature of 24 ± 1 °C, and we evaluated the biomass yields, phycobiliprotein content, and the volumetric productivity of target phycobiliproteins (phycocyanin for F2 and phycoerythrin for B1).

Pigment extraction and quantification

At the end of the two-week period, the cultures had the biomass recovered by means of centrifugation (4,000 rpm for 10 min), washed twice with distilled water and frozen at -20°C for later use. For extraction of phycobiliproteins, the stored samples were first thawed. Then, 0.02 g of wet biomass were transferred to a 1.5 mL microtube, and 1 mL of refrigerated sodium phosphate buffer (30 mM, pH 7.4) (4 °C) was added, homogenized in vortex for 30 seconds, and subjected to five cycles of freezing (24 h at -20 °C) and thawing (1 h - room temperature), with homogenization at each interval (Chittapun et al. 2020). At the end of the cycles, the microtubes were kept in a refrigerator (4 °C) overnight. Finally, the biomass was centrifuged (10,000 rpm for 10 min) and the supernatants evaluated in a spectrophotometer (Thermo Scientific, Multiskan Go model) at wavelengths of λ = 562 nm for phycoerythrin; λ = 615 nm for phycocyanin and λ = 652 nm for allophycocyanin (Arashiro et al. 2020). Additionally, the absorbance spectrum (450-750 nm) of the extracts were ranged to determine the maximum absorbance peak (Arashiro et al. 2020). A blank test was conducted using only a phosphate buffer, and the content of each class of phycobiliproteins were determined using

the adapted following equations (Bennett & Bogorad 1973), while ensuring that absorbance levels remained within the range of 0.05 to 1.0:

$$PC = (A_{615} - (0.474 \times A_{652})) / (5.34\ d_w\ O_p)$$

$$APC = (A_{652} - (0.208 \times A_{615})) / (5.09\ d_w\ O_p)$$

$$PE = \{[A_{562} - (2.41\ PC)] - (0.849\ APC)\} / (9.62\ d_w\ O_p)$$

Where: PC, APC, and PE – concentrations of phycocyanin, allophycocyanin, and phycoerythrin in dry biomass (mg g_{DW}^{-1}), respectively; A_λ – the absorbance at a given wavelength; d_w – the dry weight used for extraction (the wet biomass used on the extraction multiplied by the dry content of that sample) (g); O_p – optical pathway (cm).

Biomass yields and volumetric productivities

Total biomass production was determined based on the dry weight. For this, a sample (≈0.05 g) of the collected biomass was weighed and subsequently dried at 60 °C until reaching a constant weight and estimated the dry content of that biomass. The biomass yield was determined by the ratio of the total biomass (dry weight, g) at the end of cultivation (14 days) to the initial volume of medium (L) used in the cultivation. The volumetric productivity of each phycobiliprotein was determined from the extrapolation of its concentration, quantified from a sample of 0.02 g of the total biomass collected at the end of the 14th day of cultivation (see Pigment extraction and quantification), at the total biomass obtained in this period. Estimating this parameter allows a global assessment of the production process by relating pigment content, biomass production, and the time required to obtain it. This relationship was expressed by the following equation:

$$P_{PBP} = (PBP / b) / \Delta_t$$

Where: P_{PBP} – volumetric productivity of a strain for a particular pigment (mg L^{-1} d^{-1}); PBP – concentration of a certain phycobiliprotein (PC, APC or PE) (mg g^{-1}); b – biomass yield in dry weight (g_{DW} L^{-1}); Δ_t – time variation (days).

Statistical analyses

The mean values of each response variable (phycobiliprotein concentrations, biomass yield and volumetric productivity for each target pigment) of each treatment for each strain were compared using two-way ANOVA in IBM SPSS Statistic v. 28.0, considering light color and light intensity as factors. The significant differences amongst treatments were determined using Tukey test at 95% confidence interval level 5% ($p<0.05$). All experiments were performed in triplicate ($n=3$), and all data are presented as mean ± standard error.

RESULTS AND DISCUSSION

Effects of light color and intensity in the biomass yield and phycobiliproteins content of *Leptolyngbya* strains

In the experiment carried out with the *Leptolyngbya* sp.4 B1 strain, only the color of the light affected the phycocyanin levels, with red light promoting a significant increase in relation to the others (Figure 2a). For allophycocyanin (APC), there was no effect of any factor or of their combination. As for phycoerythrin (PE), the pigment of greatest interest in this strain, both the factors (light color and light intensity) and their interaction had a significant effect on the response variable. Red light inhibited phycoerythrin synthesis to the point of not being detected by the quantification method employed. The use of green light significantly increased the production of phycoerythrin. However, as with the use of white light, the increase in light intensity reduced the pigment content (Figure 2). The highest concentration of the pigment of interest for this strain (87.70 ± 3.16 mg g_{DW}^{-1}) was obtained using a lower light intensity of green light (GR25 treatment). Although the use of lighting with different colors and light intensities in the cultivation of *Leptolyngbya* sp.4 B1 promoted significant variations in the concentration of phycobiliproteins, there was no variation in the spectroscopic properties of

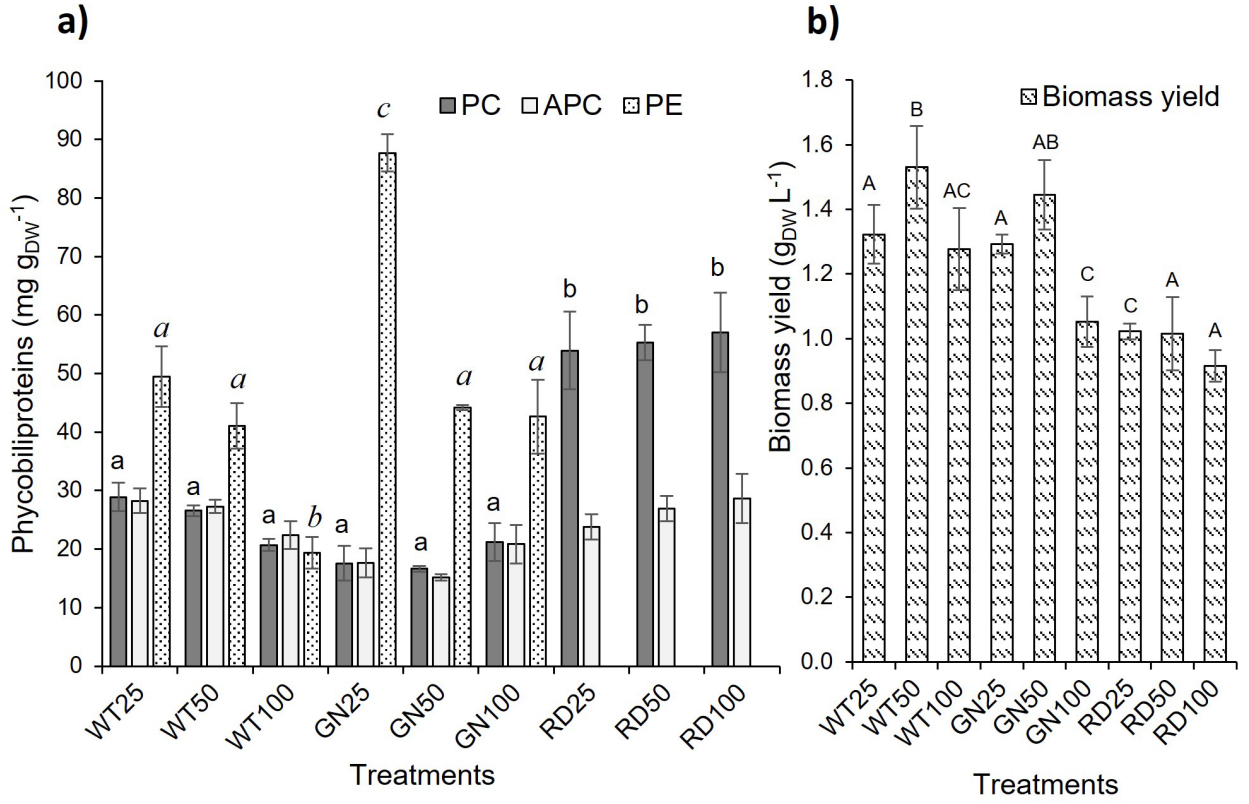

Figure 2. Responses to different combinations of light color and light intensity for the *Leptolyngbya* sp.4 B1 strain with interest in phycoerythrin production. (a) Phycobiliprotein concentration (mg g_{DW}^{-1}) after 14 days of cultivation: PC – Phycocyanin; APC – Allophycocyanin; PE – Phycoerythrin. (b) Biomass yield (g_{DW} L^{-1}) after 14 days of cultivation. The treatments are represented by the abbreviation of the color (WT: White; GR: Green; RD: Red) followed by the light intensity (25, 50 or 100 µmol photons m^{-2} s^{-1}). Data represented as mean ± standard deviation. According to Tukey's test, different letters within the same type of phycobiliprotein or biomass yield indicate significant differences ($p < 0.05$).

the extracts obtained in the different treatments, presenting peaks in the same regions, albeit with different amplitudes that indicates variations on pigments concentrations (Figure 3).

Regarding biomass yield, both factors significantly affected the response (Figure 2b). The highest yield (1.66 ± 0.13 g_{DW} L^{-1}) was obtained from the combination of white light and 50 µmol photons m^{-2} s^{-1} (WT50 treatment); an increase (WT25 treatment) or decrease (WT100 treatment) in intensity negatively

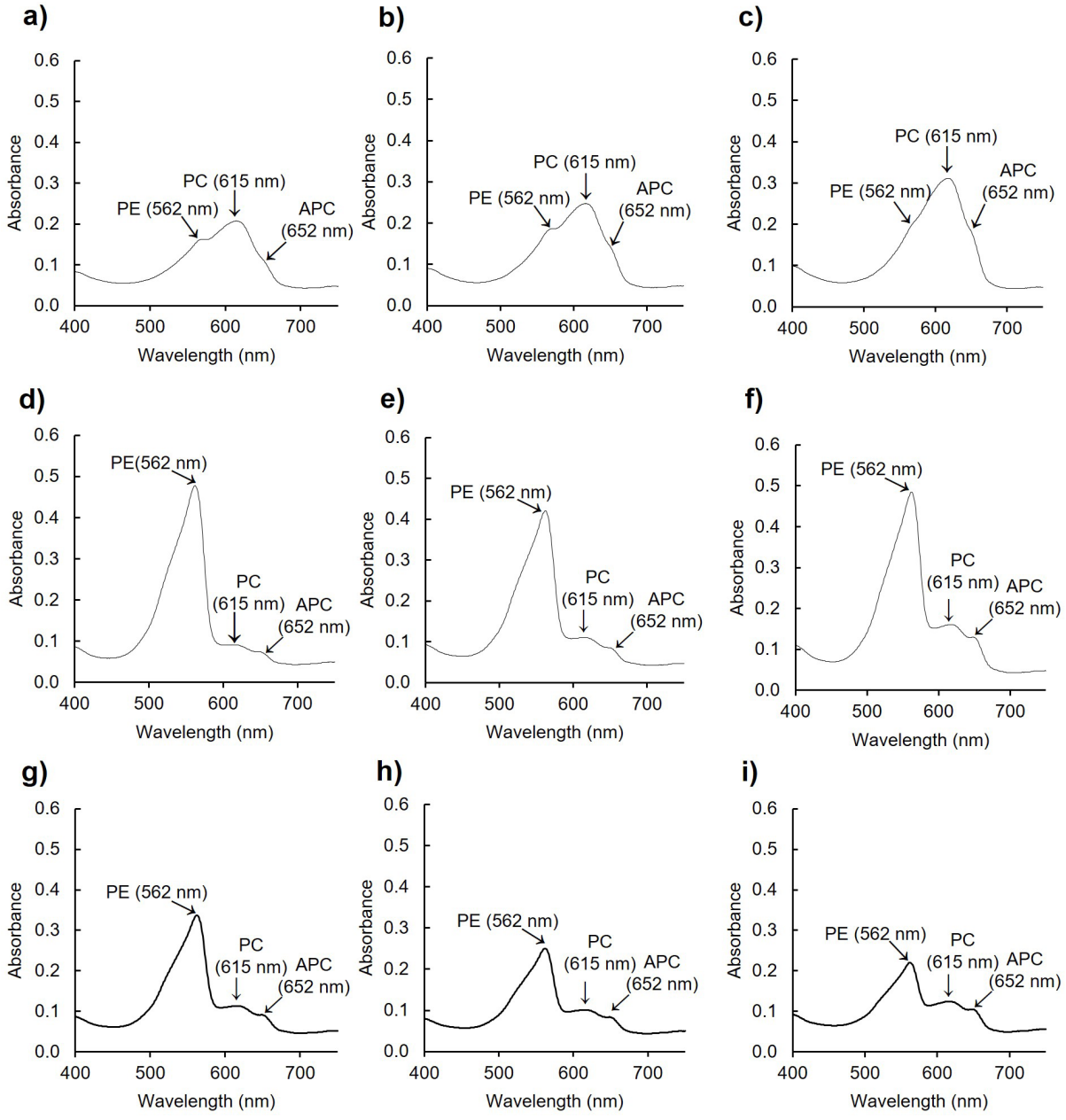

Figure 3. Absorption spectra of aqueous extracts of biomass from different treatments used in the cultivation of *Leptolyngbya* sp.4 B1. The treatments are represented by the abbreviation of the color (WT: White; GR: Green; RD: Red) followed by the light intensity (25, 50 or 100 µmol photons $m^{-2}s^{-1}$). a) RD25; b) RD50; c) RD100; d) GR25; e) GR50; f) GR100; g) WT25; h) WT50; i) WT100. PE – Phycoerythrin, PC – Phycocyanin, APC – Allophycocyanin. Data represented as average of three replicates measured at wavelengths from 400 to 750 nm.

affected biomass accumulation. Furthermore, red light reduced biomass accumulation whatever the light intensity (RD treatments). This behavior is probably explained by the composition of phycobilisome in this condition, with undetectable levels of phycoerythrin. Although there is an increase in phycocyanin production to increase energy capture under red light, this does not meet the energy requirement to reach the same biomass values obtained under white and green lights (except at GR100 treatment).

The aforementioned results are in line with those obtained by Mishra et al. (2012), who cultivated *Pseudanabaena* sp. in Erlenmeyer flasks covered with green, blue, yellow and red cellophane film. Regarding the control (white light), the increase in the phycoerythrin content obtained by the authors was 63%, while that obtained in this study was 77.42%. On the other hand, in the experiment by Mishra et al. (2012) the red filter affected the phycoerythrin concentration less intensely than the use of red light in this study. Khan et al. (2019) obtained a similar response using *Pseudanabaena catenata* USMAC16 and *P. amphigranulata* USMAC18, with higher levels of phycocyanin under red light and phycoerythrin under green light. Regarding the response to increased light intensity, studies using red algae also obtained higher production at lower light intensities (Yeh et al. 2022, Chaloub et al. 2015). This occurs due to acclimatization strategies depending on light intensity, the less light available, the greater the need for light energy collectors.

The variation in the profile of phycobiliproteins as a function of the color of the incident light occurs due to the Chromatic Acclimation (CA) mechanism, which allows cells to adapt to regulate photosynthesis according to the quality and quantity of ambient light. At least seven processes of acclimatization to green and red light are known, and in those strains originating from fresh water that present both phycocyanin and phycoerythrin, they can present three distinct responses: (i) no alteration in the pigment content; (ii) higher concentrations of phycoerythrin in green compared to red light, with no change in phycocyanin levels; and (iii) high levels of phycoerythrin in green and low levels in red light, while for phycocyanin the opposite occurs. The behavior shown by the B1 strain is compatible with the iii response, regulated by a mechanism called CA3. In the model strain *Microchaete diplosiphon* UTEX 481, the CA3 mechanism is triggered by the phosphorylation of a photoreceptor (RcaE), activated by red light, which culminates in the phosphorylation of a regulator (RcaC). This, in turn, directly controls the repression of the operon that activates the synthesis of subunits and the phycoerythrin chromophores (cpeC), in addition to activating the synthesis of phycocyanin subunits and the chromophore (cpc2). On the other hand, under green light, RcaC has phosphatase activity, reducing the activation of phycocyanin synthesis (Hirose et al. 2019, Sanfilippo et al. 2019).

Unlike *Leptolyngbya* sp.4 B1, which presents complete phycobilisome, with all pigments, the *Leptolyngbya* sp.5 F2 strain presented only allophycocyanin and phycocyanin in its composition. The Figure 4 shows the results from the experiments with the strain *Leptolyngbya* sp.5 F2 and can be perceived that significant differences in pigment contents were induced by the combination of factors (color and intensity of light). The blue and red lighting associated with the highest light intensities (BL100 and RD100 treatments) promoted greater accumulation of phycocyanin (Figure 4a), while the different combinations did not differ significantly, so that for white light (WT treatments), the increase in light intensity in the tested range (25 to 100 µmol photons m^{-2} s^{-1}) does not affect the content of this pigment. On the other hand, only the combination of blue light and greater light intensity (BL100

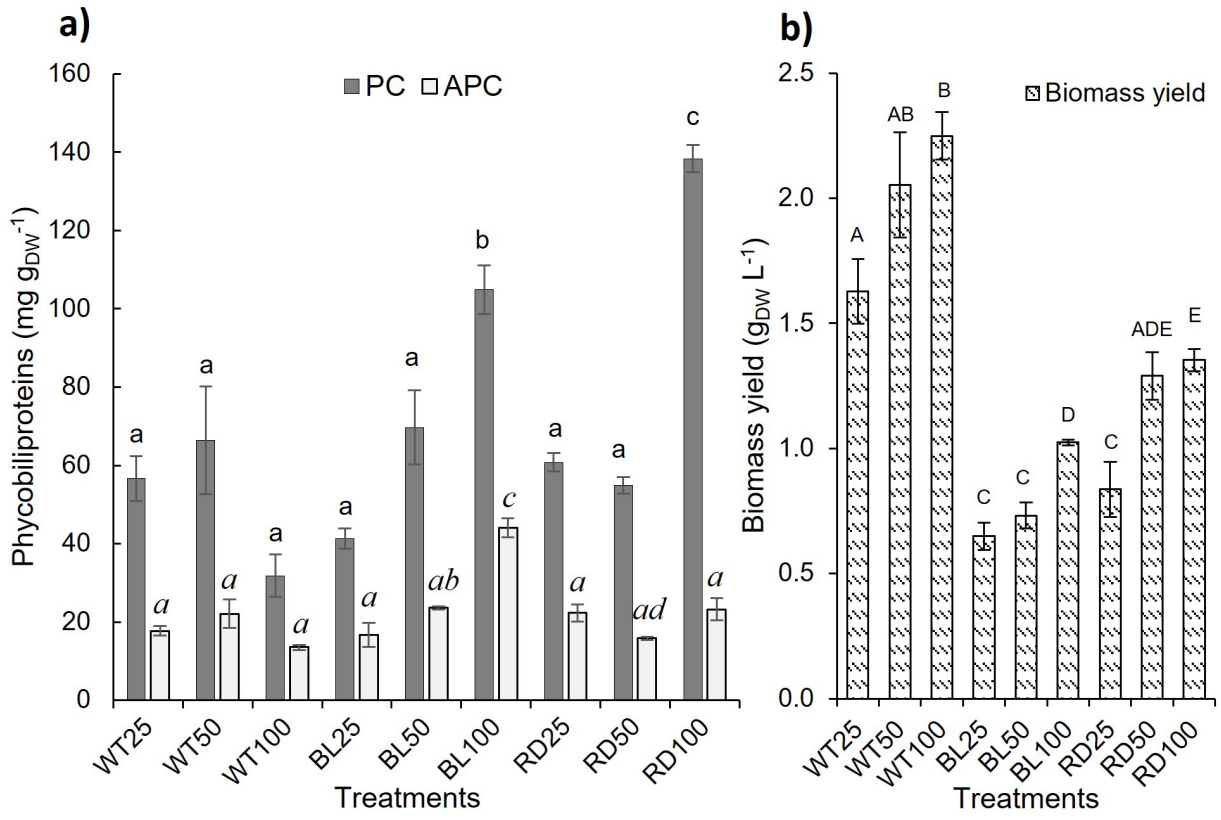

Figure 4. Responses to different combinations of light color and light intensity for the *Leptolyngbya* sp.5 F2 strain with interest in phycocyanin production. (a) Phycobiliprotein concentration (mg g_{DW}^{-1}) after 14 days of cultivation: PC – Phycocyanin; APC – Allophycocyanin. (b) Biomass yield (g_{DW} L^{-1}) after 14 days of cultivation. The treatments are represented by the abbreviation of the color (WT: White; BL: Blue; RD: Red) followed by the light intensity (25, 50 or 100 µmol photons m^{-2} s^{-1}). Data represented as mean ± standard deviation followed by letters to distinguish statistical analysis. According to Tukey's test, different letters within the same type of phycobiliprotein or biomass yield indicate significant differences ($p < 0.05$).

treatment) generates a significant increase in the allophycocyanin content (Figure 4a). The variations in the absorption spectra (Figure 5) of the extracts from biomass of *Leptolyngbya* sp.5 F2 reaffirm the variation in the concentrations of phycobiliproteins, without variation in the peaks or shoulders of each pigment, only in their amplitude.

Regarding biomass yield (Figure 4b), despite absorbing light in a smaller range than the B1 strain, which has three classes of phycobiliproteins, the *Leptolyngbya* sp.5 F2 strain obtained higher biomass yields (Figures 2b and 4b). This may have occurred because phycocyanin is more efficient in the absorption and transfer process than phycoerythrin. The effects of light color and intensity were also different between the strains. The factors color and intensity of light influenced separately. The highest nominal values were obtained in white light (Figure 4b), in which the significant difference occurred only between lower (WT25 treatment) and higher light intensity (WT100 treatment) – but not both in relation to the average intensity (WT50 treatment). In cultivation with blue light, biomass yield was influenced only by higher light intensity (BL100 treatment). In the red light, the light intensity had an effect from 50 µmol photons m^{-2} s^{-1} (RD50 and RD100 treatments) increasing the biomass (Figure

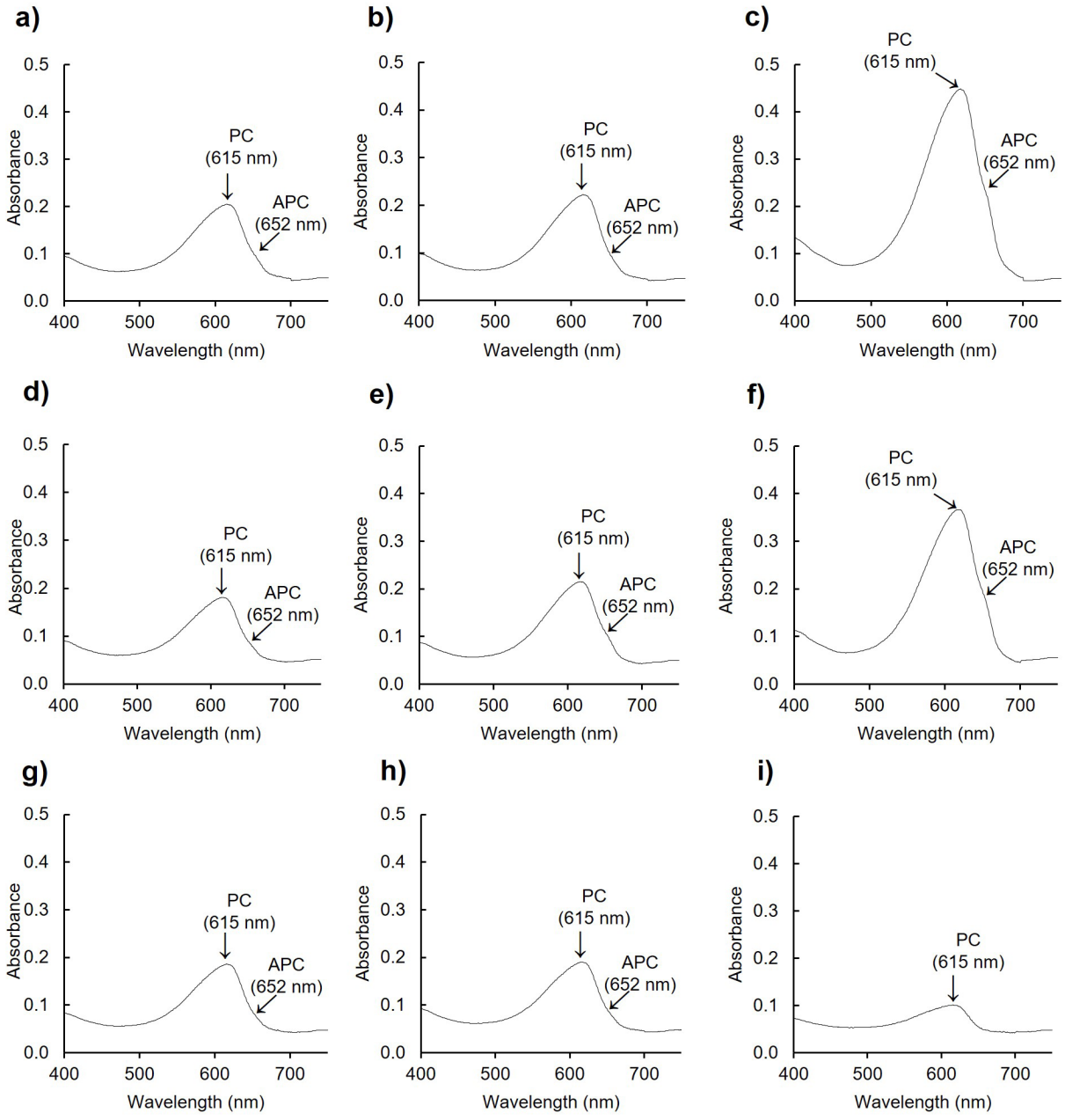

Figure 5. Absorption spectra of aqueous extracts of biomass from different treatments used in the cultivation of *Leptolyngbya* sp.5 F2. The treatments are represented by the abbreviation of the color (WT: White; BL: Blue; RD: Red) followed by the light intensity (25, 50 or 100 µmol photons m^{-2} s^{-1}). a) RD25; b) RD50; c) BL100; d) BL25; e) BL50; f) BL100; g) WT25; h) WT50; i) WT100. PE – Phycoerythrin, PC – Phycocyanin, APC – Allophycocyanin. Data represented as average of three replicates measured at wavelengths from 400 to 750 nm.

4b). Although this light color promotes average biomass accumulation, the pigment content is the highest among all treatments.

Compared to the literature, the CA on the *Leptolyngbya* sp.5 F2 strain has some similarities with some strains. In the cultivation of *Arthrospira platensis* M2, blue light promoted less accumulation of biomass (less than 1 g_{DW} L^{-1}) and higher phycocyanin content (about 132 mg g_{DW}^{-1}) compared to

white and orange lights (Zittelli et al. 2022). Similar responses were obtained in the cultivation of *Synechococcus* sp. PCC 6715 under blue light, although the levels obtained under red light were higher than those under white light (Klepacz-Smółka et al. 2020). On the other hand, in an experiment using *Spirulina* sp. LEB 18, the highest accumulation of phycocyanin (126.39 mg g_{DW}^{-1}) was under green light, but with the highest biomass under red light (1.77 g_{DW} L^{-1}) (Prates et al. 2018). In other studies, the responses to different colors of light depended on the integration of factors such as medium supplementation, temperature, photoperiod, and, mainly, which strain was being used (Ho et al. 2018, Lee et al. 2017).

In any case, the increase in phycocyanin content in cultivation under blue light compared to white light obtained in this study (Figure 4) is possibly explained by an energy compensation strategy. Because photosynthesis in cyanobacteria is dependent on the excitation of Photosystem II (PSII) by the phycobilisome, blue light, which is outside the optimal absorption range of phycobiliproteins, is not able to drive the photosynthesis through PSII (Maurya et al. 2023). On the other hand, blue light is efficiently absorbed by Chlorophyll *a*, which is most abundant in Photosystem I (PSI). As PSI is two to five times more abundant than PSII in cyanobacteria, and blue light makes it more active, an imbalance in the flow of electrons occurs (Luimstra et al. 2018). To compensate for this imbalance, cells increase phycocyanin synthesis to try to capture more light and produce more electrons and protons from PSII enabling the production of both ATP and NADPH necessary for growth. Therefore, even with a high content of this pigment, growth is still lower than in other lights (Zittelli et al. 2022). The results when cultivated under red light (Figure 3) may be associated with the classic mechanisms of chromatic acclimatization, mentioned above. However, those mechanisms have only been studied in strains in which both phycocyanin and phycoerythrin are detected, something that does not occur in any treatment.

Regarding light intensity, after a certain point (which depends on each strain), increasing or reducing intensity has no significant effect on phycocyanin production until it induces photoinhibition. Khazi et al. (2021) tested seven light intensities from 40 to 200 µmol photons m^{-2} s^{-1} in *Euryhalinema* sp. and *Desertifilum* sp., with the highest biomass and phycocyanin yields (1.21 ± 0.2 g_{DW} L^{-1} and 123.4 ± 1.0 mg g_{DW}^{-1}, and 1.18 ± 0.02 g_{DW} L^{-1} and 103.4 ± 1.0 mg g_{DW}^{-1}, respectively for each strain) obtained in 60 and 80 µmol photons m^{-2} s^{-1}, respectively. The authors found no significant difference between higher light intensities. Furthermore, as in the works by Montero-Lobato et al. (2020) and Chaiklahan et al. (2022), light intensities from 100 µmol photons m^{-2} s^{-1} of white light resulted in lower pigment concentration in *Chroococcidiopsis* sp. and *Arthrospira platensis* PB, respectively. On the other hand, data from this study (Figure 3) do not follow this trend when using blue and red lights.

Best conditions for obtaining the target pigments

Optimally, in phycobiliprotein production, high biomass yield and pigment content should be achieved in the shortest possible time. Therefore, decision-making in selecting the best treatment for producing phycocyanin or phycoerythrin may be guided by volumetric productivity (Hsieh-Lo et al. 2019). Experiments with *Leptolyngbya* sp.4 B1 strain showed that GR25 treatment resulted in significantly higher volumetric productivity of phycoerythrin (8.30 ± 0.48 mg L^{-1} d^{-1}) than other treatments (Figure 6a). This productivity was 71.13% higher than the control treatment (WT25 treatment). Despite not promoting the greatest biomass accumulation, this treatment had almost

twice the phycoerythrin content compared to the other treatments. The productivity of treatment GR25 exceeded that of strains with high phycoerythrin content, such as *Scytolyngbya* sp. LKK05, *Nostoc* sp. SW02, and *Leptolyngbya* sp. SCOM01, which contain 9.20%, 16.20%, and 5.66% phycoerythrin, respectively. Although these strains have a high pigment content, their biomass accumulation does not exceed 0.71 g_{DW} L^{-1} in 21 days at usual cultivation conditions (Limrujiwat et al. 2022). On the other hand, a phycobiliprotein productivity optimization research with *Chroococcidiopsis* sp. ranges similar

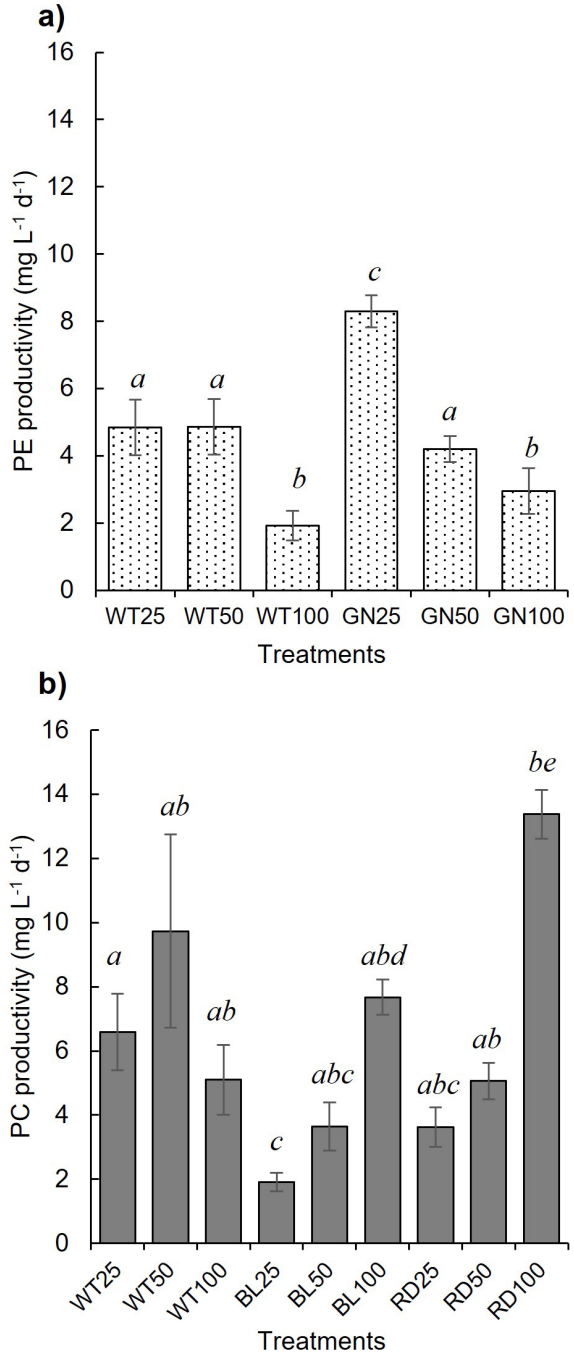

Figure 6. Volumetric productivity of phycoerythrin (PE) from *Leptolyngbya* sp.4 B1 (a) and phycocyanin (PC) from *Leptolyngbya* sp.5 F2 (b), under different combinations of light color and light intensity. The treatments are represented by the abbreviation of the color (WT: White; GN: Green; RD: Red) followed by the light intensity (25, 50 or 100 µmol photons m^{-2} s^{-1}). Data represented as mean ± standard deviation. According to Tukey's test, different letters within indicate significant differences ($p < 0.05$).

results: 9.98 mg L^{-1} d^{-1} of phycoerythrin at 70 µmol photons m^{-2} s^{-1} in BBM medium supplemented with nitrate (9 mM) (Montero-Lobato et al. 2020).

Phycoerythrin is not produced on a large scale and what is available on the market is normally produced from *Porphyridium purpureum* strains (microalgae), which has phycoerythrin content ranging from 5 to 10% (w/w) and productivity of about 16 mg L^{-1} d^{-1} under exhaustively optimized conditions (Li et al. 2022). The quality and intensity of light are only two factors that affect the productivity of this pigment, and the study of other factors such as the source and concentration of nitrogen can cause B1 phycoerythrin productivity to approach or even exceed the levels of *P. purpureum*. Additionally, because they are different pigments, although of the same class, the B1 phycoerythrin may present biological activity and applicability distinct from that of the microalgae (Husain et al. 2022).

Unlike strain B1, where the highest productivity occurred at the lowest light intensity, *Leptolyngbya* sp.5 F2 achieved greater volumetric productivity (13.38 ± 0.77 mg L^{-1} d^{-1}) at the highest light intensity under red light, the RD100 treatment (Figure 6b). However, this value did not differ significantly from the productivity at WT50 treatment (9.74 ± 3.01 mg L^{-1} d^{-1}) (Figure 6b). Therefore, either WT50 or RD100 treatments can be used to obtain phycocyanin. Although the productivity increased, it is only slightly over a tenth of the productivity achieved by commercial-scale *Arthrospira platensis* strains (Ho et al. 2018, Zittelli et al. 2022, Xie et al. 2015, Yu et al. 2019). However, this is the first step in a long optimization process towards phycocyanin production from a Brazilian biodiversity strain. In this way, one can aim to establish sophisticated and high-productivity production systems like those existing in countries such as Ireland, Israel, and Italy, in which indoor production strategies are used that take advantage of both natural and artificial light to modulate the composition of the cultivated microorganisms (Contreras-Ropero et al. 2022).

In conclusion, our results demonstrate the relationship between color and light intensity and their significant effects on phycobiliprotein productivity and biomass in local *Leptolyngbya* strains. Red and blue light promoted a significant increase in phycocyanin levels, while green light significantly increased phycoerythrin production. White light stimulates biomass production while colored lightning increases the pigment content. The highest concentration of phycoerythrin was obtained using a lower intensity of green light. Although different lighting treatments promoted significant variations in the concentration of phycobiliproteins, there was no variation in the spectroscopic properties of the extracts obtained, indicating variations only in pigment concentrations. Overall, the study contributes to understanding the effects of light color and intensity on the growth and production of phycobiliproteins in Brazilian *Leptolyngbya* strains.

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