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Enhancing growth, phycoerythrin production, and pigment composition in the red alga *Colaconema* sp. Through optimal environmental conditions in an indoor system

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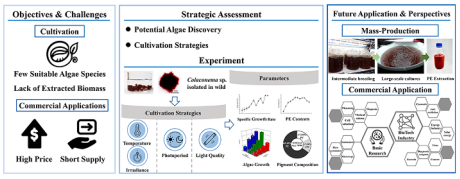
HIGHLIGHTS

- Strategic culture conditions were used to improve PE extraction.
- Algae growth increased 7–9 fold after optimization of the culture system.
- A maximum 9–10 mg g^{-1} TPBP could be obtained.
- PE accounts for up to 60%–65% of TPBP during strategic cultivation.
- Strategic cultivation supported the technology's economic feasibility.

GRAPHICAL ABSTRACT



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ABSTRACT

Phycoerythrin (PE) is a compound with strong potential for both basic research and industrial applications, but short supply and high prices have so far hindered its development. One common problem is a shortage of biomass for extraction. The aim of the present study was to determine a cultivation strategy (optimizing temperature, irradiance, photoperiod, and light quality) to produce greater biomass and higher PE concentrations in the alga *Colaconema* sp. We found that an optimized culture process could increase algae growth 7–9 fold while allowing extraction of 9–10 mg g^{-1} total phycobiliproteins, containing 60%–65% PE. Low energy costs make this approach economically feasible and competitive when compared with existing methods. Our results suggest an improved strategy for the large-scale production of PE and offer valuable applications in the algae industry.

1. Introduction

As the oldest photosynthetic light-harvesting pigment, phycobiliproteins exist in red algae, cyanobacteria, and cryptomonads (Chaloub et al., 2015). This complex compound consists of subunits of diverse phycobiliproteins and polypeptides and is located on the basal side of the thylakoid membrane in red algae. The various forms of phycobiliproteins can be identified by their colors, which are due to the presence of four isomeric metal-free linear tetrapyrrole chromophores (bilins) covalently attached to cysteine residues of apoproteins by polypeptides

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(Becker et al., 1998). In general, phycobiliproteins consist of phycoerythrin (PE, λ: 540–570), phycocyanin (PC, λ: 610–620), and allophycocyanin (APC, λ: 650–655) with different spectral properties (MacColl and Guard-Friar, 2018). These water-soluble biochemical compounds have the capability to harvest electromagnetic radiation in a spectral region and can then transfer the energy to the reaction center, providing an extra light absorption probability for chlorophyll-a (Chl-a, λ: 450–670). The pathway leads from PE to PC, APC, and then to Chl-a (Anderson & Grossman, 1990).

Studies in the phycobilisome field have progressed in the decades since 1965 (Gantt & Conti, 1965). PE is biologically active and has been reported to have high potential to generate anti-oxidative, anti-viral, anti-tumor, immunity-enhancing, and anti-inflammatory effects, rendering it suitable for applications in food, cosmetic, pharmaceutical, nutraceutical, dye, molecular biology research, and biomedical industries (Li et al., 2019a). In addition to these applications, current novel research is exploring the use of PE in the solar power industry to improve the photoelectric conversion efficiency and capture low light underwater (Li et al., 2019b; Puzorjov & McCormick, 2020). However, critical factors restrict the commercial development of this pigment, such as the lack of mass-production and its high price. At present, PE is mainly used as a fluorescent tag in flow cytometry and fluorescence microscopy, and as a photosensitizer in cancer therapy (Li et al., 2019a).

Currently, a limited number of red algae and microalgae species such as *Porphyra haitanensis*, *Porphyridium purpureum*, *Bangia fusco-purpurea*, *Synechococcus leopoliensis*, and *Spirulina platensis* are being used to drive commercial applications of PE production (Chaloub et al., 2015). The key factors are cultivation thresholds in indoor systems, productivity, the difficulty of extraction due to high polysaccharide content, and PE stability (Nguyen et al., 2017; Sekar & Chandramohan, 2008). Some research has tried to use other potential species, such as *Rhodomonas* sp. (Chaloub et al., 2015), *Phormidium* sp., *Lyngbya* sp., and *Halomicronema* sp. (Parmar et al., 2011). The microalgae species listed above are used for PE extraction to simplify the extraction process because they lack a cell wall, are easy to mass produce, and are simple to collect in a photoreactor system (Chaloub et al., 2015). However, in the commercial market, PE extracted from macroalgae still dominates. In view of this, the plan to identify a new valuable macroalga and investigate the optimal culture conditions may be more consequential in providing PE production for the global market requirements (Green-Gavrielidis & Neefus, 2016).

The extraction process is a crucial factor in the efficient derivation of undegraded phycobiliproteins. Previous studies have shown that phycobiliproteins can be stably maintained at < 60 °C and between 3.5 and 9.5 pH (Galland-Irmouli et al., 2000), and multiple solvent and physical crushing methods have been designed on that basis. Phosphate buffer, sterile water, and seawater are commonly used as solvents for these water-soluble biochemical compounds (Sudhakar et al., 2015). Methods such as homogenization, maceration in liquid nitrogen, freeze-grinding, freeze–thaw, or ultrasonication crush the cell wall to obtain the target proteins (Pereira et al., 2020).

Accumulating evidence suggests that the phycobiliproteins that form in algae are not composed of only one type of pigment in either red algae or macroalgae (Chaloub et al., 2015; Xu et al., 2020). The PE content and the variation of the biliproteins in phycobiliproteins are not only significantly affected by environmental factors, such as temperature, irradiance, photoperiod, and light quality, but also by nutrients, such as phosphate and nitrate concentration (Chaloub et al., 2015; Chen et al., 2011; Xu et al., 2020). To establish a stable algae culture system for pigment extraction, investigating the optimal culture conditions for potentially valuable macroalgae in indoor systems is crucial (Pereira et al., 2008).

To address the above problems comprehensively, this study proposed a strategy to optimize the growth, PE biosynthesis, and pigment composition of *Colaconema* sp.. The target alga is an isolate from south Taiwan and belongs to the red algae group, but it is not one of the main commercial species used in PE production at present. However, our prestudy analyzed the composition of this alga in the wild and determined the protein content to be 31.93% (dry weight). Our investigation showed that the protein (% of frond) of this alga was not inferior to the reference species currently used in pigment extraction, which have reference protein content values (as percent of dry mass) such as 33%–47.5% for *Porphyra tenera* (Galland-Irmouli et al., 1999; Sánchez-Machado et al., 2004), 20.0% for *Grateloupia turuturu* (Galland-Irmouli et al., 1999), and 8%–35% for *Palmaria palmate* (Fleurence, 1999). In view of this, *Colaconema* sp. shows a high value and potential for further applications (Cian et al., 2015).

The phycobiliprotein content of *Colaconema* sp. was approximately 6–6.5 mg g^{-1} (dry weight), and at least 50% was PE. This alga could be stably cultured in a man-made culture system. These advantages suggest that the mass-production and PE extraction from ***Colaconema*** sp. have potential applications in the commercial market. However, this alga has rarely been studied, and there are only 51 strains recorded worldwide (Guiry & Guiry, 2021). The main references for *Colaconema* sp. focus on its morphological and molecular characterization, rather than its cultivation and applications. Following the clarification of the basic physiological features this alga, it remains necessary to elucidate its physiological performance (growth and pigment accumulation) to develop future applications.

The objective of this research was to provide a new potential resource species and maximize its effectiveness in PE production for commercial applications. Our results should not only increase the diversity of algae species available for PE extraction, but also enable a mass-production culture strategy to extract competitive amounts of PE, address supply shortages, and lower the current high prices. To this end, we evaluated optimized strategies to promote algae growth and PE biosynthesis under a series of environmental conditions (temperature, irradiance, photoperiod, light quality).

2. Materials and methods

2.1. Preliminary data and algal collection

The endophytic filamentous red alga *Colaconema* sp. was found attached to the locally occurring macroalga *Sarcodia suae* in an intertidal zone in Wan-li-tong, Pingtung, Taiwan. It was isolated and maintained with Provasoli's enriched seawater (PES) medium in an environment regulated by thermostat (Tominaga, Taipei City, Taiwan).

2.2. Experimental design

The alga was cultured in incubators (Tominaga, Taipei City, Taiwan) at 20 ± 1 °C, 60 ± 10 μmol photons m^{-2} s^{-1} (light-emitting diode, LED white light), and a 16:8h light:dark photoperiod in a 5 L beaker with 800 mL of sterilized seawater (30 psu) for several days to obtain a working sample of up to 20 g (wet weight). After that, algal weight was determined after centrifugation at 2000 rpm for 5 min at room temperature (Allegra X-12R; Beckman Coulter, La Brea, CA, USA). In sequence, three aspects of culture conditions were tested (step 1: both temperature and irradiance; step 2: photoperiod; and step 3: light quality) to assess the algal growth and PE production and quality and to determine the optimal operation method that would produce the highest levels of PE.

2.3. Experimental design and culture conditions

Algae samples weighing 0.5 ± 0.01 g (wet weight) were placed in a 1 L beaker. To each beaker, 800 mL PES medium made with sterilized seawater was added. The algae were first grown in a two-variable experiment with combined effect of temperature (14 ± 1 °C, 18 ± 1 °C, 22 ± 1 °C, and 26 ± 1 °C) and light intensity (20 ± 10 μmol photons m^{-2} s^{-1}, 50 ± 10 μmol photons m^{-2} s^{-1}, and 100 ± 10 μmol photons

m^{-2} s^{-1}) under a 12:12 h light:dark photoperiod using LED white light. After obtaining the best temperature and light intensity conditions, the further effect of adjusting the light cycle was determined with a one-variable experiment that tested 8:16, 12:12, and 16:8h light:dark photoperiods. Next, the determined best conditions were used to determine the effect of monochromatic light quality in a one-variable experiment with white, red (640–680 nm), green (490–530 nm), and blue (420–460 nm) light. The light sources were light-emitting diodes (LEDs; Everlight, New Taipei City, Taiwan), and a spectroscopic analyzer (Asensetek, New Taipei City, Taiwan) was used to accurately confirm the required illumination level. A moderate amount of pumping (0.5 L min^{-1}) and rolling were provided during incubation. All experiments were conducted in triplicate and each experiment ran for 14 days. The algal weight was measured every three days when the seawater was replenished. On the 15th day, the algae growth (wet weight), specific growth rate (SGR), and pigment composition, including phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC), and total phycobiliproteins (TPBP), were measured.

2.4. Algal growth and biochemical composition analysis

2.4.1. Determination of algal growth and specific growth rate

The algal weight was determined after centrifugation at 2,000 rpm for 5 min. The SGR was expressed as % day^{-1} and calculated as follows:

$$\text{SGR (\% day}^{-1}) = 100 \ln (L_t/L_0)/t$$

where L_0 is the initial wet weight (g), L_t is the final wet weight (g) after t days, and t is the time in days. The algal structures produced during the cultivation process were weighed using an AB204 electronic balance (Mettler Toledo, Columbus, OH, USA).

2.4.2. Determination of pigment composition

PE, PC and APC concentration were determined using the spectrophotometric method. Samples of 0.1 g (wet weight) were collected in 2-mL centrifuge tubes containing 1 mL freshly prepared Na-phosphate buffer at pH 6.0 and an ionic strength of 0.1 M. Then, 0.3 g ruby sand (MP Biomedicals, Santa Ana, CA, USA), and 10 2-mm abrasive beads were added and the samples were homogenized using a high-speed homogenizer (FastPrep-24 5G; MP Biomedicals, Santa Ana, CA, USA). The algae were crushed at a vibration rate of 7 ms^{-1} for 20 s before centrifugation at 4 °C and 4,000 rpm for 20 min. The above step was conducted at least three times in succession to ensure the complete dissolution of phycobiliproteins into the solvent. The supernatant was collected and measured at room temperature according to a previously described method (Bennett & Bogorad, 1973). The PE, PC, APC, and TPBP contents were calculated individually as follows, and further, the ratio of each pigment within the TPBP contents was calculated:

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

$$\text{PC (mg mL}^{-1}) = \frac{OD_{615} - 0.474(OD_{652})}{5.34}$$

$$\text{APC (mg mL}^{-1}) = \frac{OD_{652} - 0.208(OD_{615})}{5.09}$$

$$\text{TPBP (mg mL}^{-1}) = PC + APC + PE$$

2.5. Statistical analysis

We conducted analysis of variance (ANOVA) tests to further analyze the data using SAS 9.4 (SAS Institute, Charlotte, NC, USA). Two-way ANOVAs were conducted to identify significant interaction effects of seawater temperature and irradiance on growth and TPBP, PE, APC, and PC content. One-way ANOVAs were used to test for the significance of the effects (α: 0.05) of photoperiod and light quality on growth and TPBP, PE, APC, and PC content. Where significant differences were identified by the ANOVAs, we used Duncan's New Multiple Range Test to compare the means across the treatment conditions. Data are expressed as mean ± standard deviation.

3. Results and discussion

3.1. Effect of temperature and irradiance on the growth and pigment composition of Colaconema sp.

Both temperature and irradiance are significant for the stimulation of algae growth and metabolism (Kim et al., 2019; Wei et al., 2013; Zhang et al., 2012). The temperature mainly impacts the metabolic rate, biomass composition, and biological activity (Kim et al., 2019). The growth rate, biochemical compound accumulation, and pigment production, as well as photosynthesis, are mainly affected by irradiance (Wu et al., 2015; Zhang et al., 2012; Zou & Gao, 2010). In order to investigate the combined effect of temperature and irradiance on *Colaconema* sp. weight and pigment production, the alga was cultured in an indoor system at 14, 18, 22, and 26 °C and exposed to irradiances of 20, 60, or 100 μmol photons m^{-2} s^{-1}. Growth factors, including weight and SGR, were significantly affected by both temperature and irradiance (Fig. 1, Fig. 2a-b, Fig. 3a-c, Table 1). For the treatments at 14, 18, and 22 °C with the three irradiances, higher growth was detected at higher temperatures. At 26 °C, the growth performance was inhibited by 100 μmol photons m^{-2} s^{-1}.

Our results revealed that the optimal culture conditions for *Colaconema* sp. are between 18 and 26 °C with the growth trend showing a positive correlation with irradiance. In contrast, at 26 °C, growth inhibition was detected with the highest irradiance of 100 μmol photons m^{-2} s^{-1}, while the treatments at 14 °C showed a large standard deviation and no significant effect on growth. Previous studies have revealed that temperature and irradiance have a comprehensive influence on algae growth and development. Due to the antagonism of these factors, when the temperature is high, a relatively low irradiance is optimal for growth, and, at high irradiance, the most suitable temperature is lower (Wei et al., 2013). This study found that the growth progressively declined at 26 °C with higher irradiances, but that growth was positive correlated with irradiance at 18–22 °C, revealing that the combination of these factors was adjustable. Other algae seem to have similar optimal culturing conditions, such as *Grateloupia acuminate* at 20–25 °C (<25 °C) with 20–60 μmol photons m^{-2} s^{-1} (Wei et al., 2013), *Porphyra umbilicalis*

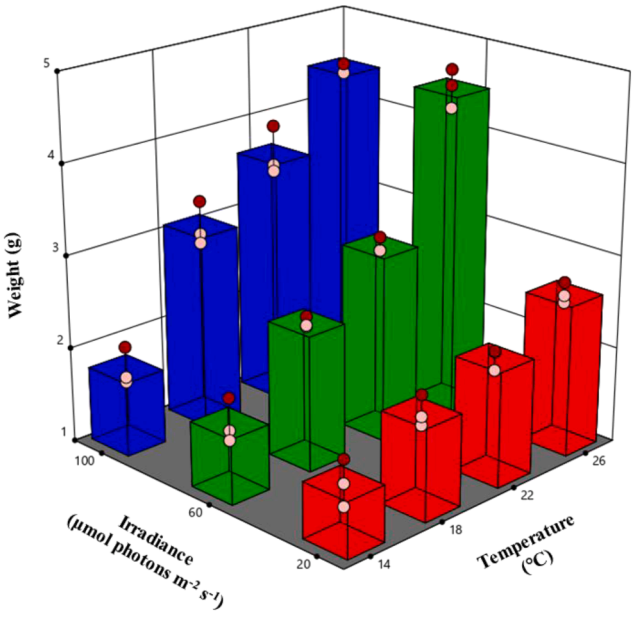

Fig. 1. Effect of temperature (14 ± 1 °C, 18 ± 1 °C, 22 ± 1 °C and 26 ± 1 °C) and irradiance (20 ± 10 μmol photons m^{-2} s^{-1}, 50 ± 10 μmol photons m^{-2} s^{-1}, and 100 ± 10 μmol photons m^{-2} s^{-1}) on the growth (wet weight) of *Colaconema* sp. over 15 days of cultivation with a 12:12 h light/dark cycle. All experiments were conducted in triplicate. Data are shown as mean ± standard deviation.