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Effect of Protocatechuic Acid on *Euglena gracilis* Growth and Accumulation of Metabolites

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Abstract: The development of efficient, environmentally friendly, low-cost approaches used to boost the growth of microalgae is urgently required to meet the increasing demands for food supplements, cosmetics, and biofuels. In this study, the growth promotion effects of protocatechuic acid (PCA) in the freshwater microalga *Euglena gracilis* were confirmed for the first time. PCA is a simple phenolic compound derived from natural plants and has a range of biological functions. The highest biomass yield, 3.1-fold higher than that of the control, used at 1.3 g·L⁻¹, was obtained at 800 mg·L⁻¹ of PCA. The yields of the metabolites chlorophyll a, carotenoids, and paramylon in the presence of PCA at 800 mg·L⁻¹ were 3.1, 3.3, and 1.7 times higher than those of the control group, respectively. The highest paramylon yield was achieved at a lower dosage of PCA (100 mg·L⁻¹), which is considered to be feasible for economic paramylon production. The growth and biosynthesis of metabolites stimulated by phytochemicals such as PCA could be an efficient and cost-effective strategy to enhance the productivity of microalgae in large-scale cultivations.

Keywords: *E. gracilis*; protocatechuic acid; photosynthetic pigments; paramylon

1. Introduction

Microalgae are considered to be one of the most promising feedstocks for the sustainable production of commodities such as biofuels, foods, cosmetics, and pharmaceuticals [1,2]. *Euglena gracilis* is a freshwater flagellate which absorbs light energy and converts it into chemical energy, besides taking up nutrients from the environment. This alga has considerable potential value because of the compounds it produces, which include vitamins, proteins, β-1,3-glucan (paramylon), essential amino acids, and fatty acids [3]. Paramylon, particularly, shows great potential as a stimulator of the immune system and has anti-tumor and antioxidant effects that may be valuable in biomedical applications [4]. The production of paramylon by *E. gracilis* must be improved to meet the industrial demands, because it naturally has a low biomass yield. Various approaches for optimizing *E. gracilis* cultivation have been trialed in the past decade, including modifying the alga strains by genetic engineering [5,6], but technical difficulties remain. Previous studies have successfully addressed the improvement of biomass and metabolite production of microalgae using genetic engineering [7,8]. However, this approach is complicated and costly. The addition of stimulating substances to the culture medium to promote the growth of microalgae has also gained the interest of researchers. Some studies have increased the biomass yield of *Scenedesmus* spp. and *Chlamydomonas* spp. by adding chemical fertilizers [9]. However, the use of chemical fertilizers causes several problems, including issues of cost and environmental pollution. Some organic sources, such as glucose, fructose, and maltose, can enhance the growth of microalgae [10,11]. These sources, however, can easily be used by bacteria, which

increases the risk of contamination of the medium. Thus, the exploration of more eco-friendly, cheaper, and safer sources to promote microalgae biomass growth is necessary to achieve their economically feasible bio-production.

Some phenolic compounds have been used as growth promoters for microalgae, owing to their wide distribution and antibacterial effects. Enhanced biomass production after exposure to different phenolic compounds has been reported for several algal species, including *Chlorella vulgaris* and *Ankistrodesmus braunii* [12,13]. In our previous research, we found that ferulic acid had positive effects on the growth of *E. gracilis*, increasing its growth by 2.5 times with respect to that of the control group when supplied at 500 mg·L⁻¹ [14]. Protocatechuic acid (PCA) (3, 4-dihydroxybenzoic acid) has been detected in the ferulic acid metabolic pathway in some microorganisms including *Margarinomyces heteromorpha*, *Margarinomyces mutabilis*, and *Pullularia pullulans* [15,16]. We therefore suspected that PCA could also be involved in the ferulic acid metabolism of *E. gracilis*, possibly leading to the promotion of growth.

PCA, a dihydroxybenzoic acid derived from fruits, green tea, and some kinds of Chinese herb, has a variety of biological effects because of its antioxidant activity. Previous researchers have reported that PCA has many benefits for humans and animals, such as anti-inflammatory, anti-hyperglycemic, and potential cancer chemo-preventive activities related to the chelation of transition metals, scavenging of free radicals by donating hydrogen atoms, and upregulation of enzymes related to the neutralization of free radicals [17]. A recent study found that PCA prevents cognitive impairment caused by amyloid β -induced Alzheimer's disease by inhibiting oxidative stress and inflammation and can therefore be regarded as a protective agent against Alzheimer's disease [18]. These potential pharmacological benefits in humans and animals have been reported both in vitro and in vivo. However, the effects of PCA on microalgae has not yet been fully explored.

This study aimed to investigate the effects of PCA on the growth and metabolism of *E. gracilis*. Cellular growth, morphology, and the accumulation of metabolites such as photosynthetic pigments and paramylon were examined.

2. Materials and Methods

2.1. Microorganism and Culture Conditions

E. gracilis Klebs strain (NIES-48) was obtained from the National Institute for Environmental Studies, Japan, and grown in Cramer–Myers (CM) medium with the following composition (mg·L⁻¹): (NH₄)₂HPO₄, 1000; KH₂PO₄, 1000; MgSO₄·7H₂O, 200; CaCl₂·2H₂O, 20; FeSO₄·7H₂O, 3; MnCl₂·4H₂O, 1.8; CoSO₄·7H₂O, 1.5; ZnSO₄·7H₂O, 0.4; Na₂MoO₄·2H₂O, 0.2; CuSO₄·5H₂O, 0.02; Vitamin B12, 0.0005; and Thiamine HCl, 0.1. The initial pH of the medium was 6.9. The CM medium, except for the organic substrates, was sterilized by autoclaving at 121 °C for 20 min. The organic ingredients in the medium and a PCA (Wako, Japan) stock solution were separately sterilized using 0.45 μ m filters (Nalgene, Thermo Fisher Scientific, Waltham, USA).

E. gracilis was incubated until it reached the exponential growth phase, at which time 10 mL aliquots of cell suspension were inoculated into Erlenmeyer flasks. Different concentrations of PCA solution were added to the CM medium, with a final culture volume of 100 mL. The final concentrations of PCA for the treatment groups were 0, 10, 100, 500, and 800 mg·L⁻¹. The cultures were incubated at 25 $\pm$ 1 °C with a 5000 light intensity (12/12 h light–dark cycle). The flasks were shaken three times per day by hand to ensure that light was provided equally to all cells and to prevent the cells from clustering.

2.2. Growth Measurement

Cell number was determined using a counting chamber (Hirschmann, Thoma, Germany) with a light microscope (Motic, BA210, Fukuoka, Japan). For cell counting, 300 μ L of the sample was taken from the cell culture, and then, 20 μ L of ethanol was added to fix the cells. The cell dry weight was measured at day 24, at the end of the log phase. To measure the cell dry weight, 5 mL of the culture was

centrifuged at 5000 rpm for 10 min. Cell pellets were re-suspended in distilled water and centrifuged at 5000 rpm for 10 min to remove residual salts, followed by drying in an oven (AVO-250 N, As one, Fukuoka, Japan) at 80 °C overnight. Finally, the cells were transferred to a desiccator and cooled to room temperature. The cell dry weight was determined by calculating the difference between the final weight and the initial weight. The pH of the culture filtrate was regularly measured using a pH meter (LAQUA-2103AL, Horiba, Japan). One milliliter of cell suspension was filtered through 0.45 µm filter paper (Advantec, GC-50, Fukuoka, Japan), and the filtrate was used for pH determination.

Specific growth rate (μ) was estimated using Equation (1), and the doubling time (DT) was calculated using Equation (2).

$$\mu = (\ln(N_2) - \ln(N_1)) / (t_2 - t_1) \quad (1)$$

$$DT = (\ln 2) / \mu \quad (2)$$

where N_1 and N_2 represent the cell densities ($\text{cells} \cdot \text{mL}^{-1}$) at the beginning (t_1) and at the end (t_2) of the exponential period, respectively.

2.3. Analysis of Cell Morphology

To determine the growth status of the microalgae, morphological observations of the microalgae cells were performed using an optical microscope (Motic, BA210). More than 100 cells were recorded using the software (Motic Image Plus 2.2S) at the sixth hour of the light period. The image processing software Image J (open source) was used to quantify cell morphology by particle analysis.

2.4. Analysis of Photosynthetic Pigments

Photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, were measured according to the method of Lichtenthaler and Wellburn [19]. To measure the photosynthetic pigments, 5 mL of algae was collected using 0.45 µm filter paper, and the collected cells were washed with distilled water and then ground with glass sands in a mortar, using 80% acetone to extract the pigments. The homogenate extract was filtered again to remove cell debris and glass sands. The extract was collected in a volumetric flask, and the volume was adjusted to 10 mL using 80% acetone. The amount of photosynthetic pigments was determined using a spectrophotometer (UV-vis 1200, Shimadzu, Japan) at wavelengths of 470, 646, and 663 nm. Pigment concentrations were calculated using the following equations:

$$\text{Chl a} = 12.21 \text{Abs}_{663} - 2.81 \text{Abs}_{646} \quad (3)$$

$$\text{Chl b} = 20.13 \text{Abs}_{646} - 5.03 \text{Abs}_{663} \quad (4)$$

$$C_{x+c} = (1000 \text{Abs}_{470} - 3.27 \text{Chl a} - 104 \text{Chl b}) / 229 \quad (5)$$

where Chl a, Chl b, and C_{x+c} are chlorophyll a, chlorophyll b, and carotenoids concentrations, respectively. Abs_{663} , Abs_{470} , and Abs_{646} are the absorbances at 663, 470, and 646 nm, respectively.

2.5. Paramylon Analysis

To quantify the paramylon content, 50 mL of microalga cells were collected by centrifugation at 5000 rpm for 10 min and washed three times with distilled water to eliminate the influence of residual salts in the medium. The harvested cells were then frozen at −20 °C for 12 h and broken by sonication. The pellets were re-suspended in 0.1% (*w/v*) sodium dodecyl sulfate and 5% (*w/v*) Na_2EDTA solution. The samples were incubated at 37 °C for 1 h and then centrifuged at 5000 rpm for 15 min. The above steps were repeated until the suspension became translucent. Pellets were washed with distilled water at 70 °C three times, collected on a 0.45 µm fiber filter, and dried overnight at 60 °C in an oven. The paramylon yield was measured gravimetrically.

2.6. Statistical Analysis

All experiments were performed in triplicate, and the results were represented as mean $\pm$ standard deviation. Significance was analyzed using the statistical software SPSS version 16.0 (IBM, USA). One-way analysis of variance was used to determine statistical differences in the growth and metabolic parameters for different treatment groups, and Student's *t* tests were used for pairwise comparisons. A *p*-value <0.05 was accepted as statistically significant.

3. Results and Discussion

3.1. Growth Profiles of *E. gracilis* with PCA Treatment

The growth curves of the periodic cultures of *E. gracilis* under different PCA concentrations was presented in Figure 1. Similar growth trends were observed in all groups, and no apparent lag period were observed at the beginning of the experiment. The cells reached the stationary phase after 24 days of cultivation. PCA promoted growth dose-dependently. The cell densities of cultures treated with PCA concentrations of 800, 500, and 100 mg·L⁻¹ were 3.1-, 2.9-, and 2.4-fold higher than that of the control group, respectively, whereas the 10 mg·L⁻¹ PCA group had values similar to those of the control group.

Figure 1. (a) Growth profiles of *Euglena gracilis* cells with different concentrations of protocathechuic acid (PCA). (b) pH changes of the culture medium. Values represent the mean of three independent measurements ($n = 3$), and the error bars indicate the standard deviations ($n = 3$).

As shown in Figure 1b, the pH of the medium was almost stable within the range of 6–7, which was optimal for *E. gracilis*, during the culture period [20]. Table 1 summarizes the specific growth rate, DT, and biomass yield under different concentrations of PCA. When the PCA concentration was increased to 800 mg·L⁻¹, the cell-specific growth rate and dry biomass yield reached a maximum of 0.23 d⁻¹ and 1.32 g·L⁻¹, respectively, significantly higher than those of the control group, of 0.14 d⁻¹ and 0.43 g·L⁻¹, respectively.

Table 1. Specific growth rate, doubling time, and biomass yield of *E. gracilis* with different concentrations of protocathechuic acid (means $\pm$ SD) ($n = 3$).

Concentration (mg·L ⁻¹)	Specific Growth Rate μ (Day ⁻¹)	Doubling Time (Days)	Biomass Yield (g·L ⁻¹)
0	0.143 $\pm$ 0.027 ^a	5.007 $\pm$ 1.064 ^b	0.433 $\pm$ 0.153 ^a
10	0.139 $\pm$ 0.027 ^a	5.169 $\pm$ 1.068 ^b	0.500 $\pm$ 0.144 ^a
100	0.211 $\pm$ 0.048 ^a	3.418 $\pm$ 0.708 ^a	1.056 $\pm$ 0.102 ^a
500	0.222 $\pm$ 0.031 ^b	3.184 $\pm$ 0.477 ^a	1.044 $\pm$ 0.222 ^a
800	0.227 $\pm$ 0.019 ^b	3.074 $\pm$ 0.259 ^a	1.322 $\pm$ 0.164 ^b

^{a,b} Different letters in each column indicated differ significantly with Student's *t*-test at $p < 0.05$.

This study is the first to report that PCA significantly promoted the growth of *E. gracilis* concentration-dependently. However, previous research has shown that some phenolic acids, including caffeic acid, p-coumaric acid, sinapic acid, syringic acid, vanillic acids, catechol, and hydroquinone, inhibited the growth of blue algae (*Microcystis aeruginosa* and *Aphanizomenon flos-aquae*) and green algae (*Chlorella pyrenoidosa* and *Scenedesmus obliquus*) because they produced many polyphenol-autoxidized products and altered the permeability and lipid/protein ratio of the algae cell membranes, thereby triggering programmed cell death [21,22].

In this study, the positive effect of PCA on the growth of *E. gracilis* might be attributed to the different type of microalga and the culture conditions established in previous studies. *E. gracilis* is more resistant to an adverse environment than the abovementioned strains because it exhibits higher tolerance to metals and acids, which adversely affect the growth of algae [23]. Thus, *E. gracilis* could grow under PCA treatment. However, the phenolic activity would be influenced by the composition of the culture medium. The antioxidant activity of phenolic compounds relates to the number and position of phenolic hydroxyl groups [24]. Phenol hydroxyl can also produce quinone and superoxide anions, especially when the presence of transition metals, particularly Fe and Cu, and a high pH change its chemical properties [25,26]. The transition metals and pH of the medium in this study were different from those of the C and BG-11 medium used in other studies, which tended to be alkaline or to contain large amounts of transition metals. Therefore, different results were observed in this study.

The mechanism by which PCA promotes the growth of *E. gracilis* might be related to its chemical characteristics and the species of microalga. The structure of PCA is unstable, and the compound is easily autoxidized, since it has two hydroxyl groups in the ortho position on the aromatic ring. This instability can lead to the formation of quinone and subsequent damage to the microalga cells by reactive oxygen species (ROS) [21]. The antioxidant defense system of the microalga and the expression of related genes would be stimulated to prevent the oxidative stress caused by ROS. Previous studies have demonstrated that the production of lipids in the biomass was 26% greater than that in the control and that superoxide dismutase enzyme activity was enhanced when *Dunaliella salina* was exposed to phenol. The growth of *C. vulgaris* increased when the cells were subjected to oxidative stress [27,28]. These results suggest that triggering the antioxidant system could promote the growth of algae.

PCA may interact with endogenous plant hormones to enhance growth. The effect of plant hormones and their analogs on the promotion of the growth of various algae has previously been investigated. The biomass productivity of *E. gracilis* was enhanced by over 20% by the indole-3-acetic acid (IAA)-producing bacterium *Vibrio natriegens* [29]. In this case, the growth promotion of *E. gracilis* could be due to IAA-induced growth that counteracted the decarboxylation of IAA [30]. Previous research has shown that caffeic acid, ferulic acid, chlorogenic acid, and other polyphenols can synergistically work with IAA in the Avena curvature test. The elongation of coleoptile sections promoted by caffeic acid [31] indicates that phenolic compounds could interact with endogenous IAA and affect growth. Both phenols and IAA oxidase are widely distributed in plants. Thus, these interactions might exist in microalgae and stimulate their growth.

The promotion of the growth of *E. gracilis* by PCA could be related to the use of PCA as a carbon source. Aromatic rings can be opened by either ortho-cleavage or meta-cleavage after the formation of a 1, 2-dihydroxybenzoid moiety [32]. Earlier studies have reported that the phenols selected from olive oil mill and petroleum hydrocarbons are degraded by microalgae [13,33], providing support for the contention that microalgae catabolize aromatic compounds. Earlier research has also shown that PCA is metabolized by *Vibrio* and is finally used by microorganisms as an exogenous carbon source [34]. Therefore, it has been speculated that PCA may also be used in *E. gracilis* metabolism during growth.

One or more of the above mechanisms may be a reasonable explanation for the growth promotion of *E. gracilis* by PCA, leading to a 3.1-times increase in biomass compared with that of the control.

3.2. Cell Morphology Analysis

Cell morphology is an essential biological indicator of *E. gracilis* growth. It reflects the response of the microalga to environmental changes. *E. gracilis* cells exhibit various morphologies, such as spherical, spindle-shaped, and elongated forms, in response to different stimuli [35]. *E. gracilis* cells are spindle-shaped and motile in normal, favorable environments. However, the cells become spherical or cystic when exposed to unfavorable conditions [36]. The cell aspect ratio is calculated as the cell length divided by the cell width and indicates the shape of the alga cells. The median cell aspect ratio of the alga cultured under different concentrations of PCA in this research is shown in Figure 2. The cell aspect ratio increased with increasing PCA concentration. At PCA concentrations of 800 mg·L⁻¹, the median cell aspect ratio increased from 2.31 to 3.18 times that of the control, and the cell aspect ratios of the alga treated with other PCA concentrations (10, 100, and 500 mg·L⁻¹) were 2.33, 2.46, and 2.58, respectively. These results are within the range of values previously reported [37,38]. The increases in the aspect ratio with increasing PCA concentration was related to cell elongation, suggesting that PCA exposure provided a suitable environment for *E. gracilis* growth. The results of the cell morphology analysis are consistent with the promotion of growth.

Figure 2. Median cell aspect ratio of *E. gracilis* cultured with different concentrations of protocatechuic acid.

3.3. Photosynthetic Pigment Analysis

As shown in Figure 3a, both chlorophyll a and carotenoid content increased concentration-dependently, but no significant change in chlorophyll b content was observed. In the 800 mg·L⁻¹ treatment group, chlorophyll a was the highest, at 6.97 mg·L⁻¹, followed by carotenoids, with a content of 2.15 mg·L⁻¹, and chlorophyll b, with a content of 1.25 mg·L⁻¹, which were 3.1, 3.3, and 0.9 times greater than those in the control group, respectively. There was no significant difference in pigments' contents for the 100, 500, and 800 mg·L⁻¹ concentrations. Figure 3b shows the ratio of chlorophyll a/b (Chl a/b) per alga cell. The Chl a/b ratio of the control group was approximately 2.9. At PCA concentrations of 500 and 800 mg·L⁻¹, the Chl a/b ratios were 5.3 and 5.5, respectively.

Earlier studies have shown that chlorophyll plays an essential role in phototrophic organisms [39]. Chlorophyll a is a critical component of the light-harvesting pigment protein complex, and photosynthetic capacity is enhanced when the chlorophyll a content is increased [40], possibly leading to algal growth promotion. The Chl a/b ratio is closely related to the light-harvesting efficiency [41]. A higher ratio of Chl a/b indicates higher photosynthetic activity and faster growth of the cells. In this study, the higher Chl a/b ratio demonstrated that the PSII complex and photosynthetic efficiency were enhanced after treatment with different PCA concentrations. A similar effect was also observed when *Cyclotella caspia* was treated with gallic acid, which stimulated the algal cells to produce more chloroplasts to cope with the applied stress [42]. Carotenoids, as auxiliary pigments, also play an important role in plants and algae in absorbing light energy for photosynthesis. Increases

in the content of carotenoids, scavengers of free radicals, might also be related to the stress induced by ROS produced by PCA [43]. Polyphenol-oxidized products are known to induce the production of radicals [21]. Some carotenoids have been reported not only to scavenge free radicals directly but also to modulate the expression of genes involved in endogenous antioxidant pathways, thereby regulating physiological activities and protecting cells [44,45]. The more vigorous growth and the greater cell length reflected changes in the physiological activities of *E. gracilis*.

Figure 3. (a) Chlorophyll a, chlorophyll b, and carotenoids content of *E. gracilis* at different concentrations of PCA. (b) Ratio of chlorophyll a to chlorophyll b at different concentrations of PCA. Values represent the mean of three independent measurements ($n = 3$), and error bars indicate the standard deviation; * in each column indicates a significant difference, with $p < 0.05$, according to Student's t -test.

3.4. Paramylon Analysis

The total paramylon yield was increased by adding PCA to the medium. As shown in Figure 4a, the highest paramylon yield (55 mg·L⁻¹) was obtained at 800 mg·L⁻¹ of PCA, followed by the yields of 50 mg·L⁻¹ at 100 mg·L⁻¹ and 47 mg·L⁻¹ at 500 mg·L⁻¹ of PCA. Paramylon yield decreased to 30 mg·L⁻¹ at 10 mg·L⁻¹ of PCA, as low as that of the control group, which was 33 mg·L⁻¹. Figure 4b shows the paramylon promotion ratio per unit of PCA (%). The highest paramylon promotion ratio per unit of PCA (5%) was obtained for the 100 mg·L⁻¹ treatment group, followed by 1.2% and 0.8% at 500 and 800 mg·L⁻¹ treatment, respectively. In this study, the total paramylon yield increased by approximately 65% with 800 mg·L⁻¹ of PCA, due to the promotion of the growth of microalga biomass. However, if we compare the promotion effect per unit dosage of PCA, a 50% increment was obtained at 100 mg·L⁻¹, whereas only 11.2% and 8.1% increments were observed at the higher dosages of 500 and 800 mg·L⁻¹, respectively. Therefore, 100 mg·L⁻¹ of PCA appeared to be the optimal dosage for producing paramylon economically.

Paramylon accumulation was enhanced when carbon and nitrogen sources were added to the medium as supplements or when *E. gracilis* was exposed to unfavorable conditions. As presented in Table 2, a paramylon content nearly 1.7 times higher than that of the control group was reported under treatment with 500 mg·L⁻¹ of ferulic acid, but the effect was due to the dimethyl sulfoxide (DMSO) used as a co-solvent, which showed some inhibitory effect on the growth of *E. gracilis* [14]. It was also confirmed that the paramylon content was increased when adding other substances to the culture medium. A combination of 8.16 g·L⁻¹ malate, 10.6 g·L⁻¹ glucose acids, and 1.8 g·L⁻¹ NH₄Cl increased the paramylon content 2.0-fold; when the medium contained 17.7 g·L⁻¹ glucose, 10 g·L⁻¹ yeast extract, and some inorganic salt, the production of paramylon was increased by 1.65 folds in a heterotrophic culture [46,47]. Paramylon production required supplemental organic carbon sources, amino acids, and vitamins, which are expensive and to compete with humans for foods. In addition, these organics would increase the risk of media contamination as they are easily used by bacteria. In our studies, the high biomass and high levels of paramylon were obtained by simply adding PCA into the medium. This strategy provides a straightforward way to overcome the current difficulty in the production of both paramylon and biomass.

Figure 4. (a) Paramylon yield (mg·L⁻¹) of *E. gracilis* culture under different concentrations of PCA. (b) Paramylon promotion ratio per unit of PCA (%·mg⁻¹). Values represent the mean of three independent measurements ($n = 3$), and error bars indicate the standard deviation.

Table 2. Effect of different supplemental sources and impact of paramylon on the growth of *E. gracilis* under different culture modes. DMSO, dimethyl sulfoxide.

Supplemental Source	Culture Mode	Effect on Growth	Impact Paramylon	Ref
0.8 g·L ⁻¹ PCA	Autotrophic	3.05-fold increase	1.65-fold increase	This study
0.5 g·L ⁻¹ ferulic acid +1% DMSO	Autotrophic	2.5-fold increase	1.70-fold increase	[14]
8.16 g·L ⁻¹ malate, 10.6 g·L ⁻¹ glucose acids, and 1.8 g·L ⁻¹ NH ₄ Cl	Heterotrophic	2.6-fold increase	2.0-fold increase	[46]
17.7 g·L ⁻¹ glucose and 10 g·L ⁻¹ yeast extract	Heterotrophic	1.75-fold increase	1.65-fold increase	[47]

4. Conclusions

In this study, the growth promotion effect of the phenolic compound PCA was confirmed on *E. gracilis* for the first time. The highest biomass yield, 3.1-fold greater than that of the control, was obtained at a PCA concentration of 800 mg·L⁻¹. The yields of metabolites such as chlorophyll a, carotenoids, and paramylon at 800 mg·L⁻¹ of PCA were increased by 3.3, 2.1, and 1.7 times with respect to those of the control group, respectively. The strongest effect of paramylon on growth per unit was obtained with 100 mg·L⁻¹ of PCA, which therefore appears to be the optimal dosage for producing paramylon economically. As this study is preliminary, further investigation using intermediates or derivatives of PCA metabolism is essential to understand the effects of PCA on the promotion of the growth of *E. gracilis*.

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